STUDIES IN HETEROGENESIS

BY

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PREFACE.

THE appearances recorded and displayed in the present memoir are only capable of receiving one or other of three interpretations.

(1) It may be said that the forms of life here described, which seem to take origin from the substance of other organisms or their germs, are not alien organisms, but normal stages in the life-history of the present forms.

(2) That the resulting forms of life are due to the invasion and multiplication of parasites within what appear to be the parent organisms.

(3) That the resulting forms of life are, in reality, heterogenetic products originating from the very substance of the organisms or of the germs from which they proceed.

Examples, however, have been selected for this memoir of such a kind as obviously to eliminate the first of these interpretations. No instructed persons at the present day are likely to take this view as to the relations existing between the producing and the produced forms of life here referred to, though in regard to some very similar observations reported by earlier investigators suppositions of this kind have been frequently advanced, and that even as late as forty or fifty years ago by such an excellent observer as Pringsheim. His suppositions were unsupported, as he admitted, by any kind of proof, and he was driven to entertain them simply because the supposed 'spores' described by him were, as he thought, indisputably formed from the very substance of the organisms in which they appeared.

We are therefore forced to choose between the two remaining possibilities.

Needless to say these two alternatives have been ever before me, and if I have adopted the latter it was only after most prolonged and careful study forced me to the conclusion that the appearances I have endeavoured in some measure to reproduce by the photographs could only be explained in accordance with such an interpretation.

This interpretation cannot be displaced by mere bald denials. The probabilities of the two views must be fairly balanced, and those who are more inclined to adopt the easy and popular

hypothesis of Infection must take the trouble to show, or at least attempt to do so, in what way the appearances can possibly admit of such an interpretation, and state what, if any, parallel cases can be adduced.

In the absence of any such information, and because the appearances seem really to show the actual transformation of the substance of the matrices into the new forms of life, while they are altogether adverse to the supposition of an entry into them of minute parasitic forms which grow, which devour the original matter of the matrices, and subsequently segment into independent organisms—for these various reasons the conclusion that we have here proofs of Heterogenesis seems justified, and in fact to be the only legitimate one.

The substantiation of such modes of origin of the organisms in question—which is my main object—far transcends in importance the following out of their subsequent fate, so long as we can be reasonably certain that the first of the three possible interpretations above referred to has been legitimately excluded.

In any case it seems clear that the observations recorded in this memoir concern a series of phenomena of considerable importance and of great generality. And whichever may be the view taken as to their real significance they are clearly phenomena worthy of most serious attention.

If they are to be explained by Infection, then assuredly they reveal a new order of things, whose stages ought to be carefully worked out by those who adopt such an explanation. The field lies open to them to trace the life-histories of the products, and to show in detail how they have been able to produce such hitherto unknown and mysterious appearances in the matrices from which they issue. Let them tell us first how they would explain the replacement, in the space of three to four days, of the contents of a Rotifer's egg by 16-20 Ciliated Infusoria; or the conversion of the entire mass of such an egg into a single specimen of one of the largest of the Ciliates.

If on the other hand the verdict must eventually be, as I imagine, that the phenomena here recorded are real instances of Heterogenesis a great step will have been made in biological science, which must have a far-reaching influence in helping to explain many problems in connection with the past and present history of our globe, and which will also throw light upon more than one disputed point in medical science.

MANCHESTER SQUARE, W.
October, 1901.

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STUDIES IN HETEROGENESIS.

BY

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I USE the term Heterogenesis in the sense originally employed by Burdach as a class name for processes by which living things arise from the matter of pre-existing organisms belonging to a totally different species—the correlative term Homogenesis being used by him, as at present, for the processes by which individuals arise, in the ordinary way, from pre-existing living things similar to themselves in organisation.

Briefly, however, I would define Heterogenesis as the production from the substance of organisms or their germs of alien forms of life.

In "The Beginnings of Life" (1872), Chap. VI., I gave a brief abstract of opinions that have been held at different times on the subject of Heterogenesis; and in the second volume of that work I cited numerous observations by others, as well as by myself, in illustration of such processes. I do not propose, however, now to go over any part of this ground again, but rather to adduce new instances illustrated by new methods.

I am well aware that my observations, as well as those of others on this subject, were received by the scientific world with great incredulity. But the absurdity of many of the views promulgated on this subject by older writers in the past should not be allowed to prejudice the acceptance of phenomena which, with care and patience, any skilled investigator may easily verify for himself.

In reply to the criticisms on this part of the subject dealt with in the above-mentioned work I have never written a single line during the twenty-nine years that have intervened, though my faith in the correctness of my own observations has never wavered. I do not say that no mistakes of interpretation were made. Whose work is absolutely free from error? I maintain, however, that the great majority of my own observations on the

subject of Heterogenesis recorded in that work were accurate and well founded, as a long series of subsequent observations has conclusively shown.

Circumstances beyond my control compelled me for many years to labour in other directions. But when at the end of 1897, after thirty years' service, I resigned my Professorship at University College, and my Physiciancy to University College Hospital, I resolved to devote the energies thus freed from the strain of teaching, and all the leisure I could command, to throwing additional light upon the subject of Heterogenesis—so all-important from many points of view—in the hope, if possible, of bringing more conviction to the minds of others.

The difficulties in this task I have always fully recognised. Heterogenesis constitutes one part of the processes included under the popular but misleading term "Spontaneous Generation," and in presidential addresses of late years, as well as on other occasions, I have heard or read, again and again, that all such doctrines have been proved to be baseless—mere chimeras, in fact, founded upon careless observations and erroneous interpretations.[1]

In order in part to meet the inherent difficulties of my undertaking I recognised how great the advantage would be to call in the aid of photography. I therefore in the first place, before beginning to make fresh observations, familiarised myself with the processes of photomicrography, and have since habitually taken photomicrographs, rather than drawings, as a means of recording what I have seen. Of course there are serious difficulties with which one has to contend in so doing, seeing that so many living organisms have to be killed before they can be photographed, and that, however carefully chosen the lethal agents may be, the appearance of delicate lower animal organisms is generally more or less altered by their action—while they may be rendered altogether shapeless or even disintegrated. Then again the important objects in investigations of this sort for the most part cannot be photographed alone—they are only too often surrounded by foreign matter of different kinds, whose presence tends more or less to obscure the picture. Finally, however great may be the pains taken by the artist (and to him my best

[1] See for instance Lord Kelvin's Presidential Address to the Royal Society (*Nature*, December, 1895, p. 111); Sir William Turner's Presidential Address to the British Association (*Nature*, September, 1900, p. 442); and Lord Lister's Presidential Address to the Royal Society (*Nature*, December, 1900, p. 136). While Prof. Oscar Hertwig, addressing recently the Congress of German Naturalists at Aix la Chapelle, is said to have accentuated the fact "that progress consists not only in adding facts to our treasury of knowledge, but also in stamping out error," by remarking that "some of the biological energy of the nineteenth century had been consumed in annihilating the doctrine of 'spontaneous generation'" (*Nature*, January, 1901, p. 286).

thanks are due) it is impossible for the 'process' blocks to reproduce all the minute details which, with the aid of a pocket lens, the photographs will disclose. Notwithstanding these inconveniences it seemed better to get rid of the personal equation by dismissing mere drawings, and letting the photomicrographs, if possible, help to break down the barrier of incredulity which at present excludes any general acceptance of the truth and universality of those processes of heterogenesis by means of which, as I believe, the lower forms of life, both animal and vegetal, are ever springing up anew in countless myriads from matrices wholly unlike themselves.

It will be seen that I have been in no hurry to speak again on this subject. I have preferred to work on in silence till I had built up a body of fresh evidence of a very varied nature which, aided by the photographs, would be likely to bring conviction to the minds of those who may be capable of accepting views at variance with their preconceptions.

I propose in this communication to deal with some processes which may be considered as typically illustrative of certain forms of Heterogenesis. I shall describe (1) some heterogenetic changes occurring in the endochrome of certain large Confervoid and other Vegetal cells and Organisms; (2) others occurring in the substance of certain encysted Ciliates; and (3) others still occurring in the substance of the eggs of different kinds of Rotifers.

I. HETEROGENETIC CHANGES OCCURRING IN THE ENDOCHROME OF CERTAIN CONFERVOID AND OTHER VEGETAL CELLS AND ORGANISMS, LEADING TO THE PRODUCTION OF SPECIMENS OF AMŒBÆ, ACTINOPHRYS, MONADS, PERANEMATA, OR MONAD CYSTS.

(*a*) Transformation of the Endochrome of Large Confervoid Cells into Amœbæ.

Two specimens of mud, containing on the surface of one an abundance of Chlamydomonads, and on the surface of the other an abundance of Euglenæ, were placed in a tall glass jar provided with a cover, similar to those used by confectioners, and ordinary tap-water was poured over the mud to a height of about five inches. The cover was replaced, and as the vessel was nine inches high there was a fair air space above the fluid. The vessel was placed outside a window with a south aspect, so that it might have the benefit of the small amount of sunlight obtaining in London during the month of October, when these observations were commenced.

After eight days a green scum was found on the side of the vessel away from the light, from near the bottom quite up to

the surface of the fluid. A portion from near the surface was scraped off with a scalpel and showed at once, on examination with the microscope, several very large algoid cells (about $\frac{1}{200}''$ in diameter), some spherical and others ovoid, of a bright emerald green colour, and having very thick walls. Subsequently other smaller separate vesicles were found, as well as concatenate groups in which the cells, though otherwise of the same kind, varied much in shape. No large masses were ever found, the common arrangement being small groups of concatenate cells radiating from some one point in three, four, or more directions (Pl. I., fig. 1), while by the side of these groups separate corpuscles also occurred, and some of these latter tended to increase much in size, attaining even the dimensions above mentioned. At other times groups of fairly equal concatenate cells were seen (Pl. I., fig. 2). Some of the large corpuscles seemed to be beginning to undergo internal changes, as the green contents were arranging themselves into spherical groups, and some of those at the periphery were becoming discolourised (Pl. I., fig. 3).

I may say at once that I have failed to identify this conferva with any known species. Nothing like it is to be found represented either in Hassal's or Cook's work on "The Fresh Water Algæ," and though I have submitted photographs and specimens to Dr. Cook, which he has been kind enough to examine, he was only able to inform me that it was probably "some vegetative form of one of the Confervaceæ."

Unfortunately very soon after discovering this plant, as the level of the water was rather inconveniently low in the jar, I poured on more water and after that was unable again to find any of the very large cells. These must have been near the surface, and thus became distributed and lost by the addition of the water. I found, however, for a time, plenty of the smaller solitary cells and of the groups scattered among other contents of the scum, which included Euglenæ, Chlamydomonads, various stationary green corpuscles of different sizes, and a very considerable quantity of Oscillatoriæ.

For a week subsequently I watched in vain for any notable changes in the larger specimens of the solitary vesicles which I had recently found, but a day or two afterwards I discovered that extensive changes were taking place in various of the smaller corpuscles, composing the concatenate groups, which had been for some days on slides beneath cover glasses, and kept in pairs under large watch-glasses—distilled water being added daily to the edges of the cover glasses to replace that which had been lost by evaporation. A study of these specimens during the next three weeks revealed many important and very varied transformations of the cell contents, which may be grouped in the main under three heads, as follows:—

(*a*) The contents of some of the cells divided whilst still green (Pl. I., fig. 4, A, B), others became uniformly discoloured to a pale drab tint, and then, while gradually becoming more translucent separated into amœboid masses—sometimes few and large, at other times small and more numerous (Pl. I., fig. 4, C, D, E). These incipient Amœbæ remain motionless for a time, then extremely slow movements commence, and in a day or two they begin to emerge from the thick cyst in which they are contained, the wall of which seems to soften at some point that happens to be in close contact with the Amœbæ. There is never anything like a swarming movement with dilatation and subsequent rupture of the cell. Outside the cell the Amœbæ exhibit the usual slow movements and changes in shape.

(*b*) At other times some obscure alteration takes place in the contents of certain cells, the colour of which becomes even of a brighter green than before, and this is followed by a simultaneous separation of the entire contents into a large number of minute green, motionless corpuscles (Pl. I., fig. 5, A.). These bodies gradually become decolourised, till only a few minute green particles remain in a little amœboid sphere of colourless protoplasm. After a time, they also begin to move and make their way out through the cell wall, but quite independently of any swarming movements. Many such cells have been seen with their contents about to separate, and others with their contents actually separated into the minute green corpuscles. Many others have also been seen in which there were only a variable number left of the small colourless corpuscles containing a few minute green particles (Pl. I., fig. 5, B, C), the others having escaped from the cell.

(*c*) Again, changes of an intermediate kind have often been seen. Peripheral portions of the green contents may become partially decolourised, and then separate into large corpuscles, which gradually become more and more translucent, and appear as incipient motionless Amœbæ, containing only colourless granules, whilst the central portion of the cell contents still remains green and unaltered (Pl. I., fig. 6). Here therefore certain parts of the cell contents become animalised distinctly before others, aud such portions are generally of rather large size. Nothing like a nucleus is at first to be detected within either of these bodies, or within either of the other forms previously described.

(*d*) Finally in other of the small concatenate cells a quite different change has been seen on five occasions. Here the entire contents of the cell becomes decolourised, more and more, till at last in place of the previous bright green granular substance there is left a mass of translucent undivided protoplasm, containing only a very few small olive green granules (Fig. 7, A, in

the larger of the two cells). In two of the specimens I found such an amœboid mass making its way out through the wall of the cell (fig. 7, B, C).

Towards the latter period of my observations, when the specimens had been under a cover glass for a fortnight or more, the various changes above described were frequently seen taking place either in contiguous cells or in others situated between two healthy and unaltered cells. Very many empty cells were also seen, in which one or other of the above described changes had taken place, and from which the metamorphosed contents had subsequently emerged. Others again were seen in which after partial decolourisation had taken place the cell contents seem to have died. Such specimens, though repeatedly examined, were found to undergo no further change.

Unfortunately my supply of specimens came to an end only too soon, and thus prevented further observations. I find in my note book the following entry: "For the last week I have scarcely been able to find any of the new alga, and none of the large vesicles. What had existed has been killed or overgrown by a great development of the Oscillatoræ, among which it was found. For the last two days I have searched in vain for specimens over different portions of the surface of the glass. The whole of the scum on the sides of the glass is now also being rapidly eaten off by a number of small red worms."

But a day or two after this alga was first found three small portions of the lateral scum containing some of the comparatively large vesicles, as well as smaller concatenate specimens, were placed in a small pool of distilled water on a hollowed glass slip, and then left on the mantle-piece covered by a large watch glass. These specimens were taken up and carefully examined every two or three days, and then carefully replaced in their pool, more distilled water being added day by day. After more than three weeks, though many of the cells in the smaller concatenate groups underwent one or other of the heterogenetic changes above described, all the large cells remained bright green and healthy looking.

The three original portions of the scum had also by the frequent mounting and unmounting for microscopical examination become broken into smaller fragments, so that I was disposed simply to preserve these fragments in my cabinet as microscopical specimens. It occurred to me, however, to try the effect of keeping them longer, but continuously beneath a cover glass, and supplied with distilled water from day to day, as many of the concatenate groups had been kept. This procedure was commenced, and four days subsequently when I examined them for the second time I found that two of the large cells, whose contents had previously presented a normal appearance, had undergone very notable changes.

In one of these cells (Pl. I., fig. 8, B) the contents, still green and rather more finely granular than it had been previously, was found to have separated into several spherical masses; while in another cell by its side (Pl. I., fig. 9, A) the whole of the green contents had divided or separated into a much larger number of small green corpuscles, very similar to what was seen in some of the smaller concatenate cells. Here too the contents quite filled the cell, but in the other (Pl. I., fig. 8, B) there had apparently been some amount of shrinkage (as is not uncommon) before segmentation occurred, though this particular form of segmentation into separate large spheres had never previously been observed. There was in this cell also a certain amount of residue in the form of discoloured, yellowish granular protoplasm.

After twenty-four hours the contents of this latter cell had undergone much further change; it only partly filled the cell, and was divided now into many small green corpuscles of about the same size as those in its companion (Pl. I., fig. 8, C), and there was also a small amount of yellowish residual granular matter in part surrounding the green corpuscles. The appearance of this cell is rather imperfectly shown in the photograph, as in it the green endochrome is not differentiated from the yellow residual matter. The contents of the companion cell appeared to have undergone little or no change. At the expiration of another day there was no appreciable change in either. But two days later the contents of Fig. 8, C, seemed to have grown, since it now more completely filled the cell; while thirty-two hours later, below and to the left of this cell, several specimens of Amœbæ with green granules in their interior were seen, either motionless, or very slowly moving as is shown by the blurred representation of some of these bodies (Fig. 8, D).[1] Around the other cell also six specimens of similar Amœbæ were seen, apparently motionless (Pl. I., fig. 9, B), and, like the others, having green granules in their interior and presenting an appearance exactly similar to that of the contents of the cells. Such bodies were only found in the immediate neighbourhood of these two cells, and nothing in any way like them had previously been found beneath this cover-glass during the twelve days that the specimen had been under observation.

In spite of all presumptions to the contrary it may be said there is no evidence that these Amœbæ had really emerged from the cells. This, however, is rendered all the more certain by observations that had previously been made on two cells which

[1] That there could be no mistake as to the identity of these two cells or difficulty in recognising them again, may be gathered from Fig. 8, A, which shows their topographical relations to one another, and also to a third cell, part of which is represented in the upper left corner.

began to change two days later than those whose transformations have been above described.

These cells were found with their contents divided into small corpuscles, in much the same way as in Fig. 9, A. They were somewhat smaller vesicles, and in one of them a portion of the cell-wall was already bulged outwards and was rather thin (Pl. I., Fig. 10, A). Two days later the spherical corpuscles in the right hand cell had become rather more distinct while, in the cell to the left, half of the contents had divided into amœboid bodies with fine green granules in their interior (Fig. 10, B). Four of these Amœbæ were seen outside and above the cell, one was in the act of passing out where the cell wall had become thinned, and one large one was still within the cell. Their movements were so slow as to be inappreciable to the eye, though the lack of definition in their photographs seems to indicate that slow movements of the granules within them must at least have been taking place. Thirty-two hours later this small cell was empty except for three specimens of Actinophrys, showing delicate ray-like pseudopodia, and having the same kind of small green granules in their interior that had existed in the previously seen Amœbæ which were at first produced from the contents of the cell. Previous experience with similar organisms had taught me that it was almost useless to attempt to photograph these bodies whilst alive, because of their slight though scarcely appreciable movements; a drop of a one-sixth per cent. solution of formalin was therefore allowed to run under the cover-glass, which led, as is almost invariably the case, to retraction of the ray-like processes. The bodies thus altered are represented by the photograph (Pl. I., fig. 10, C).

As the only specimens of these Confervoid vesicles that remained in my possession were under this cover-glass, the necessary killing of the one specimen put an end to my studies in this direction.

This last set of observations, however, was very conclusive as all the stages were seen by which the contents of the Confervoid cell became metamorphosed into specimens of Amœbæ and Actinophrys. The same speedy transition between these two animal forms has been seen on many occasions previously, and it occurs with especial frequency where Amœbæ arise, as in this case, from a transformation of vegetal protoplasm.

Looking to the very thick walls of these large separate Confervoid cells the tendency with many persons would doubtless be to regard them as 'resting spores'; but the smaller cells in the concatenate groups have walls which are quite as thick in proportion to their size, and it will have been seen that the changes that take place in these smaller vegetative cells are essentially similar to those occurring in the large separate vesicles. For

among the specimens, under the cover-glass, that were killed by the formalin there were two of the large vesicles showing important changes very similar to what had been seen in some of the smaller cells. In one the contents was decolourised almost throughout to a pale drab tint, whilst it also showed signs of general segmentation. In the other all the circumferential portion of the otherwise still green contents had become converted into hyaline, very finely granular, protoplasm. This latter cell seemed therefore on the way to the production of a completely translucent mass of protoplasm, similar to what has been seen several times in the smaller cells, and which is represented in Fig. 7.

Having watched all these varied changes during a whole month taking place in specimens kept on two or three microscope slips, and beneath cover-glasses to the edges of which a drop of distilled water was added from time to time, I can entertain no doubt but that the Amœbæ were formed, as they seemed to be, by the actual transformation of the contents of the cells.

The very thick wall of the cells would of itself make it difficult for Amœbæ or any of their descendants to effect an entry into them while the walls were still unsoftened. Moreover, until the latest stages of the changes described, nothing like Amœbæ were ever seen within the cells—that is to say no bodies, small or large, composed of clear or finely granular protoplasm. Yet to account for the appearances displayed in the photographs on the hypothesis of infection, such bodies would have had to enter each cell in numbers; or, if not, as single specimens which devoured all the contents of the cell, previously to undergoing segmentation into the few or many Amœbæ that were ultimately found therein. Of the existence of either of these processes, however, there was absolutely no evidence.

What was really seen was a segmentation of the contents of the cell while still green, or after partial decolourisation, either simultaneously throughout its whole substance, or by successive stages, and the development of the segments into Amœbæ or Actinophrys; or else a complete conversion of the entire contents of the cell, without division, into a single large Amœba.

For these diverse reasons I have no hesitation in deciding against the hypothesis of infection, and in favour of the hypothesis of heterogenesis as the only adequate explanation of the phenomena described and illustrated.

Such changes, however, far from being exceptional, are extremely common in algoid cells of various kinds, in gonidia of mosses and in the cells composing their leaves, as well as in the filament cells of Chara and Nitella.

I will briefly describe and illustrate some other examples of this transformation of vegetal protoplasm into Amœbæ and other allied organisms in order to show how commonly such a change may be recognised in plants kept under unnatural conditions.

(*b*) Transformation of the Cell-Contents of a small filamentous Conferva into Amœbæ.

I will in the first place refer to a kind of change which is extremely common in the cell contents of many small Algæ, or in those of the submerged leaves of many mosses.

The small Conferva in question was growing with a specimen of Vaucheria taken from a ditch. After bringing it home it was washed very freely for a time under a fine spray of water, in order to get rid of the mud intermixed with it, and was then placed in a shallow dish where it was fully exposed to sunlight during the day. Three days later the Vaucheria was found to be dead and discolourised, and the Conferva was dying, numbers of the filaments showing such changes as are represented in Pl. I., fig. 11, though others showed a normal appearance. In the dying filaments the cell contents had separated from the cell wall and had assumed a more or less spherical form. The mass partly seen to the extreme left of A was still green, but from that onwards to the right the protoplasm was more or less decolourised. This decolourisation is seen to be still more marked in B, the contents of the cells now forming granular amœboid masses. Two of the cells in the centre are empty owing to the Amœbæ having made their way out.

Here we have in the most obvious manner the whole endochrome of each confervoid cell individualising itself, becoming decolourised, and transformed into a single Amœba, which soon makes its way out from its birth-place, and takes on its new life as a single representative of the animal kingdom. Such changes, as I have said, are extremely common.

(*c*) Transformation of the Contents of the Resting Spores of a small Spirogyra into a number of Amœbæ.

A specimen of a small Spirogyra (known as *S. quadrata*), having the lateral mode of copulation, was found in the month of April in a small pool from which most of the water had evaporated. A portion of this weed was placed in a shallow pot on the mantelpiece only just covered by water, and under a small glass shade. After about one week the weed began to die, and towards the end of the next week many of the resting spores (which survive and remain green much longer than the filaments

in which they have been formed) were seen to be undergoing the changes now to be described.

The resting spores of this species of spirogyra have an ellipsoidal shape, and on examination with the microscope their green contents can be seen to be largely composed of the nuclear-like bodies of the bands of the two cells by whose fusion the spore has been formed. These bodies may be recognised in Pl. I., fig. 12, A. In other specimens some of these bodies seem to have fused so as to give rise to a smaller number of rather larger sub-spherical masses, such as are to be seen in B. Here they do not differ much from the bodies seen in A, though in C they are distinctly different—not only less numerous but beginning to decolourise in both the specimens shown. In D the decolourisation has become more complete and the embryo Amœbæ (seven or eight in number) more distinct, and mixed only with a small amount of refuse matter in the form of reddish-brown pigment granules. In E the decolourisation of the Amœbæ is still more obvious, and two of them have made their way out, and are lying one of them above and another at the right extremity of the resting-spore. Among the many specimens I have examined one, rather larger than usual, was seen containing a dozen or even more of these Amœbæ. The wall of the spore was thinned and about to give way.

Here again the facts seem only capable of explanation on the hypothesis of Heterogenesis.

As in the case of the large Confervoid cells the masses which subsequently develop into Amœbæ seem evidently to be actual segments of the cell-contents They are not seen at first of smaller size and gradually enlarging, as Amœbæ might be expected to appear if they had entered from without and had gradually devoured the contents of the resting spore. What is really seen is that the contents of the resting spore aggregate into 6-12 green masses, whose size remains fairly constant, but whose substance undergoes a progressive decolourisation and metamorphosis, with the result that just so many colourless Amœbæ appear, and make their way out from the thinned envelope, leaving behind them only a few refuse pigment granules.

(*d*) Transformation of the Substance of Euglenæ into Amœbæ.

The production of Amœbæ from the substance of Euglenæ is a change that takes place with great frequency, both in small and in large specimens, when they are in an encysted condition. In the former case only one to three Amœbæ may be produced, but in large specimens as many as ten or twelve may be produced within a single Euglena. After their production the Amœbæ remain as motionless spheres, it may be for two or three weeks,

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gradually increasing in size, while the original Euglena cyst slowly disintegrates or softens, and disappears.

If a portion of a Euglena pellicle is kept for a week or ten days in the dark, this kind of transformation is very commonly to be seen in some of them.

The photographs which I have selected for illustrating this process were taken from Euglenæ obtained about the middle of last September from the border of a small lake at Loughton, where they formed a pale but bright green coherent film. After they had been kept indoors in a dim light for ten days such changes as I am about to describe were occurring simultaneously in hundreds of them.

The first indication of this change is represented in Pl. I., fig. 13, A, where in the Euglena to the left, with contents granular but still green, there is an indication of some large spherical bodies forming within, while in the specimen to the right the bodies are more distinct and partly decolourised. In the three specimens shown in B, the whole substance of the Euglenæ is seen to have become converted into these bodies except for a small amount of refuse granular matter of a red-brown colour. In the specimen to the right the decolourisation of some of the spheres is more complete—their substance being homogeneous, and as yet unlike that of an Amœba. Fig. 13, C, represents one of these Euglenæ that was photographed ten days later, and it will be seen that while the cyst wall has become more indistinct the Amœbæ have become much more definite, the granules between them having for the most part disappeared, while their own substance has become much more granular—probably in part owing to their having swallowed the pigment granules previously in contact with them. After another ten days, in the last remnants of the original Euglena pellicle, I found the specimen represented at D, in which the Amœbæ have distinctly increased in size, while there are only very faint indications of the outline of the Euglena in which they had been formed. Some specimens like this I have seen slowly changing in form, and beginning to take on an active existence. In E the lowest of the Amœbæ will be seen to have undergone fission, and this is the only instance in which I have ever seen evidence of fission in these motionless Amœbæ while still within the Euglenæ. The one to the right of that in which fission has occurred showed a distinct nucleus.

Sometimes in an old Euglena pellicle I have seen some of these organisms decolourising and undergoing transformation as a whole into a single Amœba, leaving a residue, contained within its body, of brownish black pigment particles, while the cyst wall softens and gradually disappears. Three of such bodies are shown in Pl. II., fig. 14, A, each of them containing much pigment.

In B there are two specimens to the left more fully formed, containing less pigment, and in which the original Euglena cysts have almost completely disappeared, while the one on the right was free and moving slowly, so that it had to be killed with a dilute formalin solution before the photograph could be taken. Its outline is thus much more definite. This kind of change is analogous to that represented in Pl. I., fig 7, as occurring in the Confervoid cells—the whole substance of the cell in each case being converted into a single Amœba.

(*e*) Transformation of the Substance of Euglenæ into Flagellate Monads.

The transformation of Euglenæ into flagellate Monads is a rare change, and one which I have only met with on two or three occasions. It occurs within encysted organisms. An early stage of such a change is seen in Pl. II., fig. 14, C, in the organism on the left, while fully formed and slowly moving Monads were present in the Euglena on the right. Others have been seen also with more active Monads within. The substance of the Euglena becomes decolourised and then separates into small spherical bodies which, without increase in size, subsequently appear as delicate hyaline Monads, similar to those that so commonly develop from encysted Amœbæ in the filaments of Nitella and Vaucheria.

(*f*) Transformation of the Substance of Euglenæ into Peranemata.

The change of Euglenæ into Peranemata has been followed on several occasions, but I have never seen such multitudinous examples of this transformation as occurred in the pellicle of Euglenæ brought from the small lake near Loughton—when thousands were seen undergoing this change, just as others equally numerous became converted into Amœbæ in the manner shown in Fig. 13. These two changes occurred during the same period, being first recognised about ten days after the pellicle had been obtained—the Euglenæ being all encysted. The two transformations sometimes occurred side by side, but the change into Amœbæ took place most abundantly in a portion of the scum which was floating on water in a bowl, while the change into Peranemata was most common in portions of the scum which had been placed in a shallow saucer. In each case they were floating on ordinary tap water.

The beginning of the change into Peranemata is shown in Pl. II., fig. 15, A. The Euglenæ become granular and partially decolourised, and careful examination shows at first a dim indication of spherical bodies such as are to be seen in the left hand

specimen. These become rather more defined as decolourisation progresses, as is shown in its companion. The spheres are seen in B to become gradually more and more defined. These particular embryo Peranemata are distinguished from the embryo Amœbæ from the first by the number of refractive particles seen in their interior, which seem to enlarge as development proceeds. Such biscuit-like particles are particularly distinct in the right hand specimen of B, but are also obvious in C—both in the encysted Peranemata and in the two free specimens. These latter were active, owing to movements of their long and stout flagella, before they were rendered motionless by a weak formalin solution, which unfortunately had the usual effect of causing the flagella to be retracted or curled under the body of the organisms.

It will be observed that here, as in the case of the Amœbæ and of the Monads derived from the substance of Euglenæ, the spheres which become organised into Peranemata on their first appearance already fill the Euglenæ, and that in the process of organisation they neither increase appreciably in bulk nor in number. These facts are in accordance with the hypothesis of heterogenesis, and are equally adverse to the hypothesis that the Peranemata are products of infection.

These organisms are, however, sometimes produced from small encysted Euglenæ in a very different manner, and they then commonly have quite a different appearance, owing for one thing to the absence of the large biscuit-like particles which were so distinctive in those last referred to. The encysted Euglena becomes decolourised, except for some few green, brown, or yellow pigmentary aggregates, or perhaps wholly decolourised. Then the entire mass, without segmentation, after a time becomes converted into a Peranema, which gradually begins to revolve within its cell. At other times a single segmentation occurs and the products become converted into a pair of Peranemata. In Fig. 15, D, one of these small encysted Amœbæ is seen on the left; next to it another is becoming decolourised, and in the specimen on the right the contents of the cyst have been converted without remainder into two Peranemata, except that in one of them the pigment of the eye-speck was in part left. These bodies I watched for some time actively moving within their cyst, and the long stout flagellum of each was distinctly seen before their movements were stopped by a weak solution of formalin. I have also watched many others and have seen them emerge from their cysts as rather large, fully-formed Peranemata. Many of them have small, differently coloured pigment masses within, which are small unconverted, or partially converted, portions of the substance of the Euglenæ from which they have been formed. They are not like Amœbæ, and never seem to take masses of

food stuff into their bodies. At other times the work of conversion has been more complete, no coloured masses are seen within, and no large corpuscles, so that, though rather rarely, their body substance may merely contain fine granules.

(*g*) Transformation of the Substance of Vaucheria Resting Spores into Amœbæ.

The transformation of Vaucheria resting spores into Amœbæ is one which I have only seen on a few occasions, so that as yet I have not been able to trace all the stages of this change in a complete manner.

In Pl. II., fig. 16, A, one of the thick-walled and very large resting spores of *V. Dilwynii* is represented. It was filled with the usual light-green, fatty-looking corpuscles. In B another of these bodies is seen which is now crowded with motionless, resting Amœbæ, the largest one below showing a distinct nucleus. They were decolourised, but mixed up within the cyst with refuse granular pigment.

In C another smaller resting spore is seen full of much more rudimentary spherical masses, whose substance was distinctly and coarsely granular, rather than composed of clear protoplasm as in the nucleated specimen above referred to. These specimens were found in the midst of a mixture of Vaucheria and Spirogyra filaments, and the smaller one was kept under a cover glass for three days in the hope that development of the organisms might proceed, distilled water being added day by day. It is rare, however, for these changes to proceed much under such unfavourable conditions. The photograph D was taken at the end of this period. The spore was somewhat pressed out of shape by the daily subtraction and adding of water preceding and following examination with the microscope, but the Amœbæ had become more distinct, rather less granular, and two or three of them showed the appearance of a nucleus.

(*h*) Transformation of the Substance of Vaucheria Resting Spores into Monad Cysts.[1]

A specimen of Vaucheria terrestris, forming a thin green layer on a piece of earth was, early in the month of March, placed in a small pot with water just sufficient to cover the

[1] Similar to those described and figured by Cienkowski (*Archiv. für Mikroskopische Anatomie*, Band i, 1865, p. 203, Taf. xii., fig. 12), as stages in the life-history of *Pseudospora nitellarum*, and of which I have seen many ultimately (that is after two or three months) segment into a number of small spherical monads.

surface of the weed. The pot was placed on the mantel-piece of my study, beneath an inverted tumbler.

At first the Vaucheria grew, and many aerial filaments appeared, but in about ten days they died down. No examination was made, however, till four days later, though a little distilled water had been added from time to time to replace that lost by evaporation. Great numbers of resting spores were found mixed with the dying filaments—many green and fresh, while others exhibited the common kind of change, that is, the whole of the contents had become decolourised except for one or more central aggregations of fine blackish green pigment-granules.

After another fortnight the specimen was again examined, and then it was found that all the filaments of the weed had to a considerable extent disappeared. They were represented only by a thin greenish black film. Small portions of this taken up and teased out with needles showed remains of Vaucheria filaments, together with a very large number of resting spores in different conditions. Some were still green and healthy looking, except that their contained fatty-looking corpuscles were of a rather pale green colour. Others had undergone the common change, in the manner above referred to, but a much larger number of them exhibited the several stages of a kind of change now to be described.

They were mostly green, but their contents, instead of being corpuscular (Pl. II., fig. 17, A), were beginning to become granular (B), and then the contents showed a tendency to arrange themselves into rounded masses (C—F), which became more and more definitely spherical. Some portion of the contents in the later of these stages remained of a pale green colour, while other portions had become decolourised to a kind of pale drab hue (the two states being quite irregularly intermixed). In other specimens the granular contents remained wholly green till a late stage in their process of transformation.

In either case the tendency seen in very large numbers of these resting spores was for their contents to aggregate into a large number of spherical bodies of pretty uniform size, which, without altering much in bulk, became decolourised and surrounded by a definite bounding wall, enclosing a solid aggregation of the glistening refractive granules which were originally diffused through the spore (Pl. II., fig. 18, A—E). Later, after some days, the solid aggregation of granules undergoes a progressive organisation resulting in the production of a central mass of clear, highly refractive protoplasm, with a few fine granules around, between it and the cell wall (Pl. II., fig. 19, A and E).

During the earlier period of these observations, for the most

part the whole contents of the resting spore became converted into a densely packed mass of these spherical bodies, numbering from twenty to thirty, and leaving no remainders (Fig. 18, E). Later on, when the conditions probably became less favourable, only a portion of the contents of the cell underwent this kind of transformation into about eight or ten of these spheres, lying in the midst of unconverted granular matter partly green and partly decolourised (Fig. 19, E).

In these cases the most thorough examination of the resting spores, both in their natural condition and when rendered more transparent by glycerine, revealed nothing but the spherical bodies and what remained of the original granular contents. Another most important point is that the spherical bodies are not at first diminutive, gradually attaining their ordinary size afterwards. Almost from the very first they are of one size (differing only slightly among themselves) and not appreciably altering in bulk while their organisation progresses. The exceptions to this are that in some cases the rounded masses are at first rather larger than the ordinary size, as in Fig. 17, D. They are, however, obviously formed from the very substance of the resting spores. And as further evidence in support of this view I may state that the contents of not a few of these resting spores which had become converted into green granular matter, became degenerated into a homogeneous mass of pale green oily-looking matter (Fig. 19, C), and that the same kind of degeneration occasionally overtakes the contents of these resting spores after they have already arranged themselves into the more or less spherical masses that subsequently become converted into the spheres with which we are now concerned. An early stage of such a change is represented by Fig. 19, D.

Occasionally the spherical bodies were seen to be liberated by the gradual dissolution of the original spore membrane; leaving them, when the conversion of the contents of the spore had been incomplete, imbedded in a mass of granular matter (Fig. 18, F). In two or three of such liberated aggregates of spherical bodies I have found some of these spheres represented by mere empty cysts, as in two of those seen in Fig. 19, B. In one aggregate I found four of such empty cysts, the central core of protoplasm having in each of them disappeared. Evidence will presently be forthcoming tending to explain the reason of this disappearance.

Some months later I found the same kind of bodies formed within Vaucheria resting spores which had undergone the common and well known change—that is, complete decolourisation, with the exception of one or more aggregations of fine pigment granules, commonly either of a blackish green or of a red orange colour.

Early in the month of May I had in my possession a gathering of Vaucheria which after about one week was found to be swarming with resting spores. A number of these were placed in a small beaker on the end of a mantelpiece near a window, merely loosely covered so as partially to protect the water from dust. Those that were examined from time to time were found to have become decolourised and to contain near their centre an aggregation of red orange pigment granules. For long they showed only the usual varying mixtures of colourless granules and fatty looking vesicles of different sizes (Pl. II., fig. 20, A).

In examinations made, however, after eight to ten weeks had elapsed, I found that some of the resting spores had become green and had given origin to one or more filaments, both the spore and the filaments being lined with delicate, bright green chlorophyll corpuscles. I found also a fair number of the mere empty membranes of the resting spores (Fig. 20, B), and mixed with these were resting spores of the ordinary kind, except that through their granular contents one could more or less plainly discern a number of spherical bodies (Fig. 20, C, D). Other resting spores were partially empty, and contained a variable number of spherical bodies very similar to those formed so much more quickly in the still green resting spores of *Vaucheria terrestris* (Fig. 20, E).[1] An examination of different specimens of resting spores showed that these spherical bodies went through the same kind of developmental changes as the others. That is, some seemed to be composed of a mere spherical mass of granules enclosed by a limiting membrane, while others, more completely organised, showed their contents differentiated into a central homogeneous mass of protoplasm with a few small granules around (Fig. 20, F). These colourless resting spores whose changes were passed through so much more slowly, were never seen absolutely crowded with the spherical bodies, as was so often the case when the still green resting spores gave rise to them and left next to no residual matter.

Now comes the question as to the nature of these spherical bodies. Light will be thrown upon this point by a reference to analogous changes which I have several times seen taking place in Euglenæ, of the following nature.

(*i*) Transformation of the Substance of Euglenæ into Monad Cysts.

Portions of a Euglena pellicle were raised by a large section-lifter from the vessel in which it had been formed, and trans-

[1] The three or four that are seen in this figure and also in F, with no colour in their interior, are not empty spheres; they are merely spheres seen above their proper focus, their substance being much more highly refractive than those formed in the much fresher resting spores of *V. terrestris*.

ferred to a covered vase containing clear tap water. After about ten days portions of this pellicle were examined, and the Euglenæ were found to be for the most part encysted and in a resting condition, though the strong light of the lamp concentrated upon them while they were under examination or being photographed caused many of them to rotate within their cysts. Many were seen to be filled with greenish white spherical bodies at first having a granular appearance (Pl. II., fig. 21, A). As with the spherical bodies found in the green Vaucheria resting spores, they were mostly very uniform in size and seemed to be formed directly of this size by a differentiation of the substance of the Euglenæ. In no instance were Euglenæ seen containing progressively smaller bodies of like kind, as would have been the case if the Euglenæ had been infected by a number of germs which gradually increased in size till they attained the dimensions commonly seen.

Subsequently the contents of these bodies becomes more homogeneous, as may be seen in Fig. 21, B and C; while in C and D the formation of a smaller central mass of protoplasm is also recognisable. One of the masses in D has divided into two, and another seems to be beginning to undergo segmentation into such more minute Monadiform bodies as may be more plainly seen in E and F[1]. It seems highly probable, therefore, that the central protoplasm of the spherical bodies formed in the Vaucheria resting spores, and represented in Fig. 19, A, would break up into similar minute Monads, leaving such empty cysts as are to be seen in B.

Bodies of a like kind are also frequently to be met with in a double envelope Rotifer egg, which will be described later on (Pl. IV., fig. 36).

This view as to the nature of these spherical bodies is rendered all the more probable by the formation of similar spheres in the filament cells of Nitella flexilis, such as Cienkowski has figured, and such as, after they have undergone a rather long "resting period," I have many times seen resolve into a number of very minute and active Monads. These bodies, belonging to the genus *Pseudospora*, are included by de Bary in an imperfectly known group, as "doubtful Mycetozoa."[2]

(*j*) Transformation of the substance of Vaucheria Resting Spores into specimens of Actinophrys.

On two occasions in Vaucheria resting spores which had previously undergone the common kind of decolourisation with pig-

[1] In Fig. 21, D, E, F, the remains of the Euglenæ in which the spherical bodies have been formed are only very faintly appreciable—molecular disintegration having been going on for some days.

[2] "Fungi, Mycetozoa, and Bacteria," 1887, p. 446.

mentary remainders, I have ultimately found twelve or more specimens of Actinophrys rather than the Monad cysts above described. One of these two resting spores when first seen contained about sixteen specimens of Actinophrys of rather unequal size, together with a mass of olive green pigment and some scattered granular matter. Close examination showed that very slight movements of some of them were taking place, so I gave up the notion of photographing this specimen in favour of seeing what the subsequent changes would be. I put the specimen aside therefore under a large watch glass, and when I examined it twelve hours later I found only four specimens of Actinophrys left within the resting spore (together with the mass of pigment), though another of them was still just outside, having very distinct rays and moving rather rapidly. Its texture was exactly similar to that of the bodies within the resting spore. It was filled with small refractive particles, and showed a distinct nucleus. Rays could also be seen pertaining to one of the bodies remaining within the resting spore.

II.—HETEROGENETIC CHANGES OCCURRING WITHIN THE SUBSTANCE OF CERTAIN ENCYSTED CILIATA, LEADING TO THE PRODUCTION OF SPECIMENS OF PERANEMA, OF EMBRYO AMŒBÆ, OR OF MONADS.

(*a*) Transformation of the Substance of Encysted Prorodons into Peranemata, Amœbæ, or Monads.

The changes to be first described occurred in a number of encysted Ciliates which were found in a tall vessel, filled with water to the depth of about seven inches, into which six weeks previously an abundance of Chlamydomonads and Euglenæ were placed, near the end of the month of October. This vessel remained during most of this time on a stand outside a window with a south aspect. After about four weeks a brownish green scum had grown over the walls of the vessel, and two weeks later some of this scum was scraped off with a section-lifter and examined. It was found to consist to a large extent of a feltwork of reddish brown filaments (algoid), mixed with green and grey Oscillatoriæ, and a number of Euglenæ—many of these latter being elongated, and having spiral surface markings. The Euglenæ were most abundant in the scum over the lower third of the vessel, while in the upper third more especially there were found a large number of active Prorodons (with distinct terminal oral cylinders) mostly gorged with small green corpuscles; other specimens were seen becoming motionless and beginning to encyst; while a much larger number of them were actually encysted and decolourised—the cysts being very thick, plicated, and without spines or projections of any kind.

These encysted specimens were searched for and examined at intervals for a period of about three weeks, by which time the supply was pretty well exhausted. The following facts were made out.

The tendency in the great majority of them was to reach a certain stage of reorganisation, and not to advance any further; the conditions apparently not being favourable to the occurrence of the ordinary changes which these encysted Prorodons were accustomed to go through. And perhaps this might have been in part due to the fact that three or four days before the date of my first examination of the scum I had put a cover over the jar (as the surface of the water was getting covered with "blacks"), and had consequently cut off the supply of fresh air. This undoubtedly had a very injurious effect upon some of the organisms on the sides of the vessel, as over the lower third there were a number of small red worms in loose sheaths actively eating the scum before the cover was applied, but when I began my examination I found some of these worms dead within their sheaths, as well as very many of the latter empty, but not a single one containing a living worm.

The stage of organisation attained by a great number of the encysted Ciliates is represented in Pl. II., fig. 22, A, in which some differentiation of the contents of the cyst had taken place leading to the production of a large curved mass, composed of a darker and closer aggregation of granular matter than existed in other parts of the matrix. No stages between this and that of a completed organism which was subsequently produced from some of them have been definitely traced. I have seen six of these organisms in all, and they were apparently large, vigorous specimens of the genus Oxytricha, but of a kind not exactly like either of the illustrations to be found in the works of Prichard and Saville Kent. The first of them was seen about to emerge, and revolving rapidly within its cyst first in one direction for about fifteen revolutions, and then, without an appreciable interval, for about a similar number in the reverse direction. What surprised me much, however, was to see four delicate spherical bodies about $\frac{1}{3500}''$ in diameter dashed around within the cyst by the rapidly moving Ciliate. These bodies were exactly similar to what I have often seen produced from the substance of Euglenæ, and within various algoid cells, and which after a time become converted either into Monads or Amœbæ. While being dashed about as they were they would naturally appear as simple spherical bodies. Unfortunately I have no photograph of this specimen. No photograph could be obtained without the use of formalin or some such agent, and as there were other specimens beneath the same cover glass that I wished to examine in the living state I postponed this proceeding, and soon afterwards

accidentally dropped the microscope slip on to the floor, and thus unfortunately lost all the specimens that were contained thereon.

The Monads or Amœboid corpuscles must have been produced from some separated portions of the encysted mass, but the companionship of such bodies within a cyst with a developed Ciliate I had never previously seen, though associations of the same kind have been since observed.[1]

The production of this encysted Oxytricha from the substances of an encysted Prorodon was of itself sufficiently surprising, though previous observations have been made by other investigators tending to show that transformations of encysted Ciliates into other forms are by no means uncommon.[2] In regard to the reality of this particular transformation, I may say that the scum under examination contained no other large cysts, only those of the kind with which we are now concerned, and that besides the Prorodons the only other Ciliates seen during the very many examinations made were numbers of Stentors, with the exception of this Oxytricha within the cyst and five others, exactly similar, which were seen at different times, evidently recently born and in association with empty cysts (Fig. 22, B) slightly dilated and having thinner walls. I say recently born because three of these organisms contained not a particle of food within them, while each of the two others only showed two or three small fragments of green matter. Each time that I wished to photograph one of them, and drew some of a one-sixth per cent. solution of formalin under the cover glass, I was disappointed, seeing that each of the three young Ciliates under the influence of the formalin burst and became a mere formless mass of granular matter—as is very commonly the case, and especially with young specimens; while on other occasions the two remaining Ciliates could not again be found after the application of the formalin, amidst other matter that was beneath the cover glass.

I am, however, perfectly certain that these young though large Oxytrichæ seen in the free state, were similar to the active specimen for some time watched revolving within its cyst, and consider there is no room for doubt that they had recently emerged from some of the empty Prorodon cysts. But large numbers of these encysted Prorodons have been seen to undergo a totally different change, and the transformations now to be

[1] I have seen an encysted Kolpoda divided into four vigorous young ones, and within this cyst there were also three or four of these Monad-like bodies being driven about by their active companions. Other such associations will be referred to later on pp. 27 and 38.

[2] This is fully recognised in Carpenter's work "The Microscope," seventh edition, 1891, p. 707, and references to some of these transformations were given in "The Beginnings of Life," vol. ii, pp. 493-497.

described have been met with most frequently in portions of the scum which, after having been scraped off the sides of the glass, have subsequently been kept for two or three days, previous to examination, in such compressed or rolled-up masses in a small vessel containing distilled water. Other specimens, however, have also been found undergoing these changes in portions of scum examined directly after they had been removed from the walls of the glass. The most common change has been one by which the entire contents of the cyst has ultimately broken up into a number of very coarsely granular spheres which developed into **Peranemata.**[1]

During the early stages of this change the encysted matter as a whole loses its ordinary granular appearance; it becomes more refractive, and with what would be an approach to translucency were it not closely studded with coarse, fatty-looking particles (Pl. II., fig. 22, C). Soon traces of separation into distinct units begin to show themselves (Figs. 22, D, E, and 23, A), and later still spherical bodies of variable size actually separate from the parent mass, so that the whole cyst may be seen to be completely filled with a closely packed aggregate of motionless and coarsely granular spheres (Fig. 22, F).

At other times the units that separate from the matrix may be of fairly equal size and larger, but leaving a certain amount of unconverted residual matter, as in Fig. 23, B. Here the units not being so closely packed showed slow, semi-rotatory or pendulum-like movements, and had to be killed with a weak formalin solution before the photograph could be taken. In another cyst there were found six coarsely granular units varying much in size, some of which were exhibiting the same slow, semi-rotatory movements, though no flagella could be detected (Fig. 23, C).[2] Some refuse granular matter was seen among them, and it seemed probable that some of the Peranemata had escaped, as the cyst was not nearly full. A very similar condition of things existed in another specimen, only in this case the organisms exhibited rather freer movements within the half empty cyst (Fig. 23, E), and languid movements of their long flagella were distinctly seen.[3] After the application of the formalin solution these flagella, as is generally the case, were no longer visible, and are consequently not to be detected in the photograph. The typical pear-like shape of one of the free

[1] Organisms something like colourless Euglenæ, though with a much stouter flagellum, and now often described under the name of Astasiæ.

[2] A weak solution of iodine was employed in order to kill these organisms, which was not quite effectual. I thought all movements had ceased till I saw the indistinct outlines of some of the organisms in the photograph.

[3] This photograph, and also that from which A was taken, shows a greater enlargement than the others—500 diameters instead of 250.

Peranemata is to be seen in the specimen from which Fig. 24, A, is taken, together with what looks like a nucleus near its hinder extremity. In this case the long flagellum was not retracted, but it was unfortunately bent downwards under the organism, and being altogether out of focus is not represented.

In other specimens of the Prorodon cysts there has been a production of **Amœboid** corpuscles, though this change has been much less frequent than that leading to the production of Peranemata. A cyst partly full of such units is represented in Fig. 23, D. These bodies were distinctly lighter in colour than the embryo Peranemata, and they also contained only fine granules in their interior instead of coarse fatty particles or globules. Then again it must be recollected that four similar corpuscles were seen in the cyst that contained the active Oxytricha before referred to. Such bodies may develop either into Amœbæ or Monads, but judging from the developed form of very similar bodies found in the encysted Ciliates next to be referred to, and also within certain Rotifers' eggs, the probability is that they would have taken on the form of Monads.

In another of these Prorodon cysts I found six spherical bodies, each of which contained a large nucleus in its interior (Pl. II., fig. 24, C). These were clearly neither embryo Peranemata nor embryo Amœbæ nor Monads; they seemed to have more resemblance to some kind of Fungus germ, similarly formed out of the substance of the previously encysted Ciliate. Their nature must, however, at present be regarded as very uncertain.

In two other specimens some very strange and remarkable changes were seen. The encysted matrix in each case was found to be full of delicate hyaline daughter cysts, and these in one specimen contained small embryos of some kind, several of which were slowly moving, so that formalin had to be applied before they could be photographed (Fig. 24, B). Two of these spheres, having much thicker walls than the others and large stationary bodies like nuclei, bore a very close resemblance to the germ-like units found within the cyst last referred to. The other specimen is more highly magnified (Fig. 24, D); its contents were motionless and generally bore some resemblance to fungus germs in process of development. I have seen one other cyst with the same kind of contents, of which no photograph was obtained.

(*b*) Transformation of the Substance of Encysted Stylonychiæ into Monads and Peranemata.

Changes very similar in nature, though not quite so varied, have also been traced in another set of matrices which for about three months remained without showing any appreciable change;

and it was only after that period that some of them began to undergo developmental processes resulting in the production of Ciliates of the kind known as *Stylonychia lanceolata*. The fully developed matrices were contained in fairly thick cysts covered with short conical projections almost exactly like what are figured by Saville Kent in his "Manual of the Infusoria" as pertaining to one of the other representatives of the genus Stylonychia. One of these cysts, having the projections therefrom stained by a weak solution of Westphal's fluid, is represented in Pl. III., fig. 25, A. I do not propose now to deal with the question of the origin of these encysted bodies. I will only say that I have found many thousands of them in association with a gathering of Euglenæ; and though I have seen many specimens of Stylonychia come out of them I have never seen a single one of them formed by the encystment of such a Ciliate. They appear rather to have been derived from much smaller matrices, found in association with the more developed specimens, which gradually increased in size and formed a thicker envelope upon which the spinous processes were finally developed. The mature specimens varied a good deal in actual size, and they always presented a greyish colour, which contrasted notably with the silvery white appearance and smooth outline of certain encysted Vorticellæ sparingly intermixed with them.

As I have said, these matrices remained for about three months without undergoing any further change. Then, within a few of them, Stylonychiæ began to develop, to rotate, and ultimately to emerge from their prison. Fig. 25, B, represents one of these Ciliates soon after emergence, and before any food had been taken; while Fig. 25, C, represents a more developed specimen which had swallowed, and was digesting, a small Euglena. Within the latter specimen a nucleus was faintly seen just below the oral cleft, and below that was the situation of the contractile vesicle—not wholly at rest during the taking of the photograph. I may say that these photographs were only obtained after many failures, as both very weak iodine solution and very dilute formalin almost invariably caused the Ciliates to completely disintegrate in two or three minutes. At last I succeeded by using a very weak solution of Westphal's "mastzellen stain" (1 to 60 of distilled water). A drop of this solution drawn under the cover glass soon stopped the organism's actual movements of translation, though its cilia went on vibrating for fifteen to twenty minutes longer, and therefore before the photographs could be taken. I was thus more fortunate with these Ciliates than with the specimens of Oxytricha that were developed in the cysts previously referred to, with which iodine and formalin solutions only were tried.

About the same time that these normal developments of the

contents of the cysts were first observed I began also to find other cysts, in which the contents were undergoing, or had undergone, certain heterogenetic transformations. About ten weeks previously portions of the original Euglena pellicle had been transferred by a section-lifter to the surface of some clear water, and the changes now to be described occurred in small portions of this pellicle which had gradually dropped to the bottom of the vase to which the transference had been made. These mere dregs had been lying at the bottom of the vessel, about five inches below the surface of the water, for a variable number of weeks. The original pellicle having been exhausted, before emptying the vessel I was induced to examine some of this residual matter, and soon found changes going on which caused me to treasure the whole of it, and examine it bit by bit for many days. Multitudes of the Stylonychiæ cysts were there—many thousands of them—looking healthy and presenting no evidence of change in either direction, as in the lower specimen of Fig. 25, D.[1] Others were developing Stylonychiæ, many of which were seen within, emerging from, or in active movement outside the cysts. Here and there also the contents of other cysts were undergoing one or other of the heterogenetic transformations now to be described.

The first indication of an approaching heterogenetic change was similar to that occurring in the thick-walled Prorodon cysts previously referred to. The whole included substance became rather paler and more refractive, and studded with fatty-looking granules, as in the upper specimen of Fig. 25, D, and in Fig. 26, A. Then in others there was distinct evidence of the contained matrix separating into granular spheres. It seemed here again, as with the large Confervoid matrices, with the Vaucheria resting spores and with the Prorodon cysts, that the new units were not formed by the whole mass dividing into 2, 4, 8, 16, &c., but by the units merely separating more or less simultaneously from the parent mass (Pl. III., fig. 26, B, C), Other cysts were found from which many of the spheres, developed into **Monads,** had escaped, eight to twelve only being left, and these exhibiting their usual pendulum-like or slow semi-rotary movements, so that a weak iodine solution had to be employed before photographs could be taken (Fig. 26, D, E). While examining the cyst from which the former of these figures was obtained I watched one of the Monads therein divide into two. It will be seen that these Monads are of unequal size, and some of them are rather smaller than the

[1] This lower specimen of Fig. 25, D, shows the usual appearance of these cysts when no stain has been employed.

spherical masses shown in Fig. 26, B, C, a fact probably due to fission having taken place pretty generally, and to the size of the units having been thus reduced. Several of these Monads were also seen to contain a distinct nucleus. Outside the cysts they were likewise seen to vary much, both in size and shape. Some had the form of a short sausage, others were shorter still, while others had a distinct egg-like form or else were nearly spherical. They have a very slow, wobbling movement, and this is brought about by the languid action of an anterior short flagellum, while trailing behind in a straight line is a flagellum from three to four or even five times the length of the Monad itself, but having no independent movements of its own. I have made several rather unsatisfactory attempts to stain and then photograph these Monads, but Fig. 26, F, shows fairly well two small specimens with their long and short flagella, though the latter are out of focus. Both flagella are to be seen coming off, as they always do, from the under portion of the anterior extremity of the organism.

These Monads all contain rather coarse, fatty, and sometimes faintly brownish granules, and a nucleus is often distinctly visible. Some of them have even been seen at times slightly changing their form, while one that was watched became distinctly amœboid. I have been unable to find any such organisms represented either in Pritchard's or in Saville Kent's works on the "Infusoria."

Occasionally, only part of the contents of a cyst separates into Monads, the larger portion remaining as a spherical or ovoid mass. This was the case in Pl. III., fig. 27, A, in which only two moving Monads were seen together with some granular matter, probably resulting from the death and partial disintegration of others. After the application of weak iodine to check their movements, one of the two remaining Monads became disintegrated, so that only one is to be seen in the photograph. The large ovoidal mass became deeply stained of a red brown colour by the iodine solution, and is consequently seen to be very dark in the photograph.[1] It is, however, the rule that the sub-

[1] This red brown colour with a weak iodine solution is usually considered to denote an admixture of glycogen with the protoplasm. Thus De Bary, speaking of a variety of protoplasm for which he formerly proposed the name of *Epiplasm*, writes as follows: "It is distinguished from ordinary protoplasm by being more highly refringent, by its peculiar homogeneous and glistening appearance, and especially by the reddish brown or violet brown colour which it assumes when treated with very dilute solution of iodine. Errera has recently shown that this reaction with iodine is due to the circumstance that the Epiplasm contains a relatively large quantity of glycogen permeating a protoplasmic or albuminoid vehicle; the term *glycogen-mass*, or shortly *glycogen*, may therefore be substituted for that of Epiplasm" ("Fungi, Mycetozoa, and Bacteria," 1887, p. 77).

stance of these particular encysted Ciliate matrices becomes stained of a deep red brown colour on the application of this reagent. It is possible, therefore, that the large ovoidal mass contained within the cyst in question might, had it been left, have gone on to the production of a Ciliate. But the co-existence of Monads within the same cyst with a large mass from which a Ciliate might have been produced, reminds one of the four Amœboid corpuscles or Monads which I have previously described as existing in the same cyst with a very active Oxytricha (p. 21).

In other specimens embryo **Peranemata** have been found within these spinous Stylonychia cysts instead of Monads. Thus, in Fig. 27, B, six of these fatty, conglomerate bodies were tightly packed within the cyst, in association with a large ovoidal portion of the original matrix; and in Fig. 27, C, the whole matrix had become converted into similar bodies. In another specimen the whole of the matrix had also become transformed into Peranemata, though these were of much larger size; and, as the cyst was not closely packed with them, they exhibited the usual slow pendulum-like movements, previous to the application of a weak iodine solution (Fig. 27, D). These Peranemata were exactly similar to those found within the Prorodon cysts, which are represented in Pl. II., fig. 23, B, C.

Several of these Stylonychia cysts have been found greatly thickened by the formation of successive concentric layers, apparently associated with progressive shrinking of the contained matrix, which was generally reduced to about one-third of its original bulk, while presenting a distinctly Amœboid appearance (Fig. 27, E).

Several other Stylonychia cysts have been seen with their contents divided into four rather unequal spheres, having distinct bounding membranes (Fig. 27, F). These are possibly not heterogenetic products. They may be mere results of fission of the original mass and capable of developing into four small Ciliates, though the fact of their encystment within the parent cyst is rather against this notion. In all the numerous cases in which I have seen fission taking place in various kinds of encysted Ciliates the process has occurred after, or immediately anterior to, the production of Cilia, and the products of fission have invariably continued their active movements until they succeeded in bursting their cysts, and thus releasing themselves.

I have never seen anything like Fungus germs develop within these Stylonychia cysts, though otherwise the changes into Peranemata and Monads have been very similar to those occurring in the larger and thicker-walled Prorodon cysts. It is a note worthy fact, however, that none of these heterogenetic changes began to manifest themselves till the contained matrix had remained apparently quiescent for three months or more.

That we have had to do in each of these cases with actual transformations of the substance of the encysted Ciliates into Peranemata, Monads, or Amœbæ seems to me, surprising though it may be, scarcely to admit of doubt. No theory of infection by germs of Peranemata, Monads, or Amœbæ could by any possibility account for what occurs, namely, the simultaneous change throughout the whole mass of the matrix, and the separation therefrom of rather large spheres which then become organised into one or other of these forms of life. Nothing in the previously recognised life history of Peranemata, Monads, or Amœbæ will in any way explain such facts and the appearances represented in the photographs.

(*c*) Transformation of the Substance of an Encysted Vorticella into Monads (?)

A limited number of the silvery and comparatively thin-walled Vorticella cysts have been associated with the Stylonychia cysts during the whole time they have been under observation, but in only one single specimen of the former have heterogenetic changes of any kind been encountered. This was in the case of a small Vorticella cyst which was filled with 8-12 coarsely granular spheres, that appeared to be exactly similar to those which in the other two encysted Ciliates subsequently developed into Monads (Fig. 27, G). The substance of the Vorticella matrices would appear, therefore, to be more stable than that of either of the other two Ciliates; although if the changes I have described had been due to invasions from without, these particular cysts should have been the first to manifest change, looking to the thinness of their cyst walls. As a matter of fact, however, the first to yield heterogenetic products were the very thick-walled Prorodon cysts.

III. HETEROGENETIC CHANGES OCCURRING IN THE EGGS OF CERTAIN ROTIFERS, LEADING TO THE PRODUCTION THEREIN OF PERANEMATA, OF MONADS, OF AMŒBÆ, OF MONAD CYSTS, OF PRIMITIVE SPORANGIA, OR OF CILIATED INFUSORIA.

(*a*) Transformation of the Substance of the Eggs of a Species of Lepadella into Amœbæ or Peranemata.

In two collections of Euglenæ examined during September, 1900, I found a large number of extraordinary Rotifer eggs, from which projected in almost all directions numerous spinous processes of great length. I was quite unable to trace the origin of these bodies, that is, the stages by which the ordinary egg of

a Rotifer with its usually thin envelope, either within the parent or after having been laid, gradually developed such an astonishing armature of spines. The intermediate stages were practically unobserved, though there were in different specimens some considerable variations in the length of the spines, and it was noticed on more than one occasion that when one of these bodies was in part pretty closely surrounded by other matter, the spinous processes were either abortive or wanting in such parts, as may be seen in Pl. III., fig. 28, B. This certainly favours the notion that the spinous processes have been developed after the eggs have been laid.[1]

Some were found in an early stage of development (Pl. III., fig. 28, A), while in many others an embryo Rotifer could be plainly seen within, as was evidenced by its pharynx, a red eye-speck, and the play of oral cilia, all of which were distinctly seen in the specimen of which Fig. 28, B, is a photograph. It was a long time, however, before I succeeded in watching one of these embryos emerge from its envelope, and was able to photograph it. The exit of the embryo is undoubtedly hampered by the presence of the cover glass on one side and the glass slip on the other, so that three or four which I watched for long periods failed to make their exit and died exhausted. At last, however, I succeeded in witnessing the exit of one of them, and (after stopping its movements with a weak iodine solution) in getting a photograph of it. This embryo is shown in Fig. 28, C, and careful examination of it and also of others rather older soon made it plain that they were embryos of a species of Lepadella, adult specimens of which were also present in considerable numbers (Fig. 28, D). The character of the foot, of the pharynx, and oral aperture, as well as the situation and colour of the single eye-speck, made this plain; while the shield-like form was gradually assumed by the young Rotifer as development proceeded.

Many of the adult Lepadellæ were seen containing a large egg of the ordinary kind, that is, with a thin enveloping membrane free from anything like spines. It was clear, however, that the embryos which these bodies with the long spines produced were those of a species of Lepadella—we had to do in fact with Rotifer germs notwithstanding their extraordinary armature. Only one kind of Lepadella seemed to exist in association with these Euglenæ, and, when first laid, the eggs of these Rotifers were unprovided with spines of any kind. The eggs that were thus provided can, therefore, only be regarded as 'resting eggs.'

[1] I have since seen spines of this kind developing from the eggs of a smaller Lepadella, no trace of which existed when they were laid and attached to filaments of a large Spirogyra. Numbers of these eggs were seen, with spines in different stages of development.

After the bowl containing the Euglena pellicle had been brought indoors and placed under a shade on the mantel-piece for about a week, many of these Rotifer germs, under the influence of diminished air and light with somewhat higher temperature (whatever other differences there may have been), no longer developed in the proper manner into Rotifers. The embryo mass contained within the cyst underwent some peculiar change throughout its whole substance, as a result of which it ultimately divided into embryo Peranemata.

The early stage of this change, which results in the production of **Amœbæ or Peranemata,** is shown in Fig. 29, A, B, in which the mass becomes much more coarsely granular, and its whole substance becomes studded with fatty-looking particles. Another of these embryo masses which has actually divided into Amœbæ is represented in Fig. 29, C,—a nucleus in one or two of them being distinctly visible. In Fig. 29, D, complete division is seen to have been accomplished into a number of much smaller Amœbæ.

A similar division into coarsely granular Peranemata is shown in the portion of the Rotifer egg that is uncovered in Fig. 29, E. A cyst with extremely long spines is seen in Fig. 29, F, which was empty except for one large and motionless Peranema that existed in the upper part of the cyst, but which, owing to the intervening wall of the egg with its spines, is very indistinctly seen in the photograph. This figure is destined principally to show the very great development of the spines in this specimen of the 'resting egg.'

(*b*) Transformation of the Substance of the Eggs of Different Species of Diglena into Peranemata, Amœbæ, Flagellate Monads, and Monad Cysts.

Changes in the eggs of specimens of Diglena have been observed in part similar to those of the spinous cysted Lepadella. Thus an egg still within the body of a dead Diglena has been seen in the stage anterior to that of its complete division into **Peranemata** (Fig. 30, A); while another much smaller egg (Fig. 30, B), has been seen almost completely segmented into a number of these bodies, with no apparent movement, although their hazy appearance in the photograph and the lack of definition of the coarse granules seen in their interior point to slow movements of these granules, such as may occasionally be made out.

Again, another of these eggs was seen, the contents of which had divided into six large **Amœbæ**. Three of these were more superficial, and in focus, in the photograph from which Fig. 30, C, is taken; while the other three were in a lower plane, and consequently are not shown. These bodies were motionless. More

distilled water was added to the edge of the cover glass and the slip was put on one side, covered by a large watch glass. When it was next examined, thirty hours later, one of the three superficial bodies was found to have emerged from the egg, as may be seen in Fig. 30, D. This photograph was taken with a lower power so as to shorten the exposure and minimise the effects of the very slow movements of this free Amœba. Examination with a high power, however, showed in each of these three superficial Amœbæ a translucent nuclear body. The extremely slow and slight changes in form observed in the Amœba which had made its way out of the egg, causes its photograph to present a distinctly more blurred appearance than that of either of the other two, which were evidently motionless, so that in the photograph the granules in their interior are plainly visible.

A great difference in the size of the segments into which the Diglena egg separates at different times was seen here, as in the case of some of the other animal matrices whose heterogenetic changes have been previously described. This, however, was still more notable in the heterogenetic products of the large Confervoid cells shown in Pl. I., figs. 4 and 5.

Changes very similar to those already detailed, though more varied in nature, have also been observed occurring in another kind of Rotifer's egg provided with a double envelope. These bodies were first seen on November 17, 1900, in connection with a Euglena pellicle derived from a gathering obtained on October 28. They existed there in association with the spinous Stylonychia cysts, whose changes have been previously described. And they, like the spinous cysts, remained week after week without undergoing any appreciable change, though many hundreds of them came under observation, while ordinary eggs of *Diglena catellina* (which were extremely abundant in association therewith) developed embryos within a few days after they had been laid. After two or three weeks also some of these latter showed one or other of the heterogenetic changes previously described, though the eggs with double envelopes neither began to undergo homogenetic nor heterogenetic changes till near the end of the eleventh week after they were first observed. The heterogenetic changes were, in fact, first discovered, and it was only by a mere chance that I succeeded in obtaining from about the last remaining fragments of this pellicle specimens of these peculiar eggs within which developed Rotifers were seen. My surprise, however, was great when I found that the embryo which emerged therefrom was the form known as *Diglena catellina*, which had all along been so abundant in association with this stock of Euglenæ, and which had been developed so abundantly

and rapidly from ordinary eggs. The others must, therefore, be the 'resting eggs' of this species.

These eggs with double envelopes presented the following characters. They varied somewhat in size, and a good deal of variation was also seen in different specimens in the width of the space between the outer and inner envelopes. Often the space was scarcely appreciable. Fig. 31, A, shows one of these eggs in which the space between the two envelopes had about the average proportionate width. When viewed slightly above the focal distance the outer envelope had a slight but distinct bluish tint, and with a good adjustment of light there was an appearance of very delicate and close concentric markings on the surface of this envelope. At one pole, as may be seen, the two envelopes were almost always united by some intermediate substance. This outer envelope was evidently much firmer and tougher than that of the ordinary Diglena egg, and is so impermeable to aniline stains that I have seen large numbers of these eggs remain unstained after they had been for twelve hours or more in such solutions. The contents were evenly granular, though the granules were rather larger, and darker also, than those in the ordinary Diglena egg; and in none of these 'resting eggs' could I discover the clear nuclear body which can be so commonly made out in the former (*see* Pl. IV., fig. 37, A).

On several occasions I saw evidences of commencing development in these eggs of the kind that may be seen in Fig. 31, B, but no further stage could be discovered till February 2, 1901, when just before clearing out the vase which had contained this Euglena pellicle (now exhausted) I observed a small amount of still green scum on the side of the vessel just above the level of the water. In a scraped-off portion of this scum I found no less than six developed Rotifers within as many of these eggs with double envelopes. One of them was near the edge of the portion of scum, and this I determined to watch, as the others were more or less enveloped by Euglenæ, and I should have had little chance of seeing the embryo properly even if it had been able, thus embarrassed, to escape from its double chamber. In this embryo a distinct pharynx and two eye-specks were visible, and as it was motionless I photographed it at once (Fig. 31, C). The outer envelope was crumpled by the process of scraping the scum from the side of the vessel, but in some of the others among the Euglenæ the double envelopes were quite distinct, and all the embryos were fully developed.

In a very short time after the photograph had been taken, movements of the pharynx began to show themselves. I added some distilled water therefore to the edge of the cover glass, and placed the slip on the mantel-piece under a large watch glass. When, three hours later, I examined this specimen again I found

that the embryo had just emerged, as it was close to the empty cyst, and after watching its feeble movements for a few minutes and recognising that it was a Diglena, I was obliged, in order to photograph it, to sacrifice it as well as the others. This I did with a very dilute solution of formalin, and fortunately death occurred without producing any great amount of contraction of the very shadowy embryo—though its foot was tucked under and hidden by its body (Fig. 31, D). I have also added a photograph of another older specimen with food in its stomach, whose movements were arrested by a dilute solution of Westphal's 'mastzellen stain' without producing contraction of the body (Fig. 31, E), a result not easy to obtain.

Later on while studying the dregs found at the bottom of this vase I found many of these Rotifers within, and watched them actually emerging from, these resting eggs. This happened while I was studying the heterogenetic changes in other of these Rotifers, as well as in the spinous Stylonychia cysts (which were associated with them in great abundance) in the refuse matter formed by small portions of the pellicle which, during some weeks previously, had been gradually dropping to the bottom of the vessel, as already described.[1]

Many thousands of these eggs have been seen in the dregs above referred to, and the heterogenetic changes observed have been far more numerously encountered than those occurring in either of the other kinds of Rotifer eggs. The stages in the process of change leading to the production of Peranemata and Monads have also been rather more fully made out. The kinds of change observed have likewise been more numerous; since, in addition to Peranemata, Monads, Amœbæ, and Monad cysts have been seen to be produced within the substance of these eggs—and that after they had remained without change in the same fluids for periods varying from three to four months.

I have been unable to differentiate the early changes which lead ultimately to the production of Monads from those which eventuate in the production of Peranemata, but as the former have been produced from these eggs far more frequently than the latter, I shall in the first place refer to the production of **Monads.**

It is mostly, though not invariably, the case that in the eggs

[1] *See* p. 26. If the pellicle had been in the vessel in which it was originally contained, at the bottom of which there was a layer of mud, the portions of the pellicle that fell to the bottom would probably never have been examined. There is, therefore, a very distinct advantage, in these investigations, likely to result from transferring a portion of the pellicle to fresh water in a clean vessel, so that, after some weeks, portions of the pellicle that have subsided may be easily examined.

which go on to the production of one or other of these heterogenetic transformations one of the first changes that occurs is a certain amount of shrinking of the substance of the egg, and, in consequence, the production of a much greater space than usually exists between its two envelopes.

Then, also, the contained mass gradually becomes very distinctly altered in appearance. Instead of the normal evenly granular contents the whole mass assumes a more refractive and fatty appearance, and is more or less closely studded with coarse, fatty-looking granules (Pl. III., fig. 32, A). Then faint traces of the mass separating into a number of more or less spherical bodies, varying a good deal in size in different eggs, begin to show themselves, and gradually become more distinct (Fig. 32, B, C, D). How long these early stages take—whether they are slow or rapid—I have been unable definitely to ascertain, because though I have often kept such specimens for several days beneath a cover glass, briefly looking at them from day to day in order not to subject them too long to the glare of the lamp, their developmental changes have been almost always arrested. Such conditions seem altogether unfavourable for the progress of these early changes. I am strongly disposed to think, however, that they are rather slowly brought about—much more slowly than after segmentation has once occurred; concerning the rate of which latter changes I have made some definite observations that will be presently recorded.

After a time we may see the whole egg densely packed with a number of spherical bodies, as in Fig. 32, E. These were motionless, and each contained a number of fatty-looking granules, which, however, are not represented in the photograph, probably owing to slow movements of the granules occurring while the photograph was being taken. The egg itself is one in which, at this stage of the transformation, the space between the two envelopes may be obliterated.

Now I come to the description of two important specimens in which development took place while they were under the cover glass.

In Fig. 33, A, is shown one of these eggs containing about a dozen dark, granular, more or less spherical bodies. Although the large spherical bodies seemed to be quite motionless, there must, I think (as in the last specimen), have been some molecular agitations in their interior capable of moving the coarse granules they contained. These were very distinct in the specimen itself, and yet instead of them there is only a hazy appearance in the photograph. The slip containing this specimen was then left under a cover glass on the mantel-piece, the weather being very cold at the time, so that the temperature to which it was exposed was for the most part not much above 50° F. After twenty-four

hours the specimen was examined again, and I was very surprised to find how much it had changed. As with the heterogenetic contents of one of the large Confervoid cells, in the interval both growth and segmentation had taken place (*see* p. 10), so that the contents were now tightly packed, while the spherical bodies were smaller and nearly twice as numerous as they had been when last examined (Fig. 33, B). Kept under similar conditions and examined thirty hours later, I found that only a small amount of change had occurred, as may be seen by examination of the photograph that was then taken (Fig. 33, C). I imagine that the exposure to the strong lamp-light in the taking of the first two photographs, together with the confinement under the cover glass, must have nearly killed the contents of the egg. Certainly the taking of the third photograph did so, as when the specimen was examined again after another twenty-four hours, in the place of the spherical bodies I found a mere formless mass of granular matter—the spheres had all become disintegrated.[1]

The other instance of development occurring underneath the cover glass is still more interesting because it showed the nature and developmental destination of the spherical bodies into which the substance of these Rotifer eggs had been dividing. One of the eggs is shown in Fig. 34, A, divided into about sixteen closely packed, motionless, spherical bodies, containing the usual fatty-looking granules. After the photograph had been taken, the specimen was left underneath the cover glass on the mantel-piece, protected as before. It was examined again only ten hours later. The cover glass had slipped slightly in getting the excess of water away before putting the slip on the microscope, so that a foreign body to be seen near it at first, and serving to mark it, had been pushed further away. Many granular Monads were now found within the cyst having slow semi-rotatory movements (Fig. 34, B). The cyst was, in fact, now half empty, while outside, in its immediate neighbourhood, were other of these same granular Monads, having very slow, somewhat wobbling movements, which careful examination showed to be due to the swayings of a short anterior flagellum, while trailing behind, but otherwise motionless, was a very long flagellum—even four or five times as long as the Monad itself (Fig. 34, C). These Monads were indeed almost exactly similar to those that were produced within the spinous Stylonychia cysts. They showed similar variations in shape and size, and also the same tendency to exhibit amœboid changes in outline.

[1] The exposure was repeated for the third photograph, as at the first attempt, through an oversight, I gave much too long an exposure, and therefore almost immediately afterwards took another photograph, from which Fig. 33, C, was taken.

Sometimes within these double envelope Rotifer eggs very delicate **Amœbæ** have been produced instead of Monads. Thus Fig. 35, A, shows a cyst containing twelve of these bodies. The cyst was slightly compressed by accident, and this was fortunate as it has brought more of these bodies into focus than would otherwise have been the case. In Fig. 35, B, we have a representation of another specimen of the same kind, only here the cyst is half empty, and the organisms that remained were rather more fully developed. Two or three of them showed very distinct nuclei, and one of these is plainly visible in the photograph. It will be remembered that larger and more coarsely granular Amœbæ were also found in one of the Diglena eggs, as represented in Fig. 30, C, D.

The production of **Peranemata** within this kind of Rotifer's egg has only been met with on two or three occasions, and that when heterogenetic changes first began to manifest themselves in the substance of these eggs, but not subsequently. In one of such specimens, within the shrunken inner envelope, and replacing the ordinary substance of the egg, six large, conglomerate, pale drab Peranemata were seen, all exhibiting slight jerking and semi-rotatory movements. These movements were stopped by running under the cover glass a drop of a one-sixth per cent. solution of formalin, and immediately that the organisms were at rest the specimen was photographed (Fig. 35, C). The photograph has to be taken very quickly after the application of the formalin, as it speedily seems either to dissolve or to cause a rupture of the very thin envelope of protoplasm which binds together the large fatty-looking masses that enter so largely into the composition of some of these embryo Peranemata of heterogenetic origin, and gives them such a conglomerate appearance. The extent to which this disintegration occurs may be judged from Fig. 35, D, which is from a photograph of the same specimen taken only twenty minutes after the other, and in which some of the Peranemata may be seen to have become completely resolved into separate fatty globules and particles. This same kind of thing has been observed previously on several occasions, but it does not happen with the mere coarsely granular spheres which subsequently, and often after fission, take on the form of Monads.

In another specimen, in which again there happens to be scarcely any space between the two envelopes, only about half of the substance of the egg has undergone transformation into Peranemata (Fig. 35, E). The rest of the egg substance is coarsely granular, and looks as if the heterogenetic change had commenced, although from some cause it had been arrested. The embryo Peranemata were six in number, but of very unequal

size, the largest of them being to the right in a lower plane, and consequently out of focus. This partial transformation of the contents of the egg is very similar to what is represented in Pl. III., fig. 27, A, B, which shows partial transformation of encysted Stylonychiæ, the products in the one case being Monads and in the other Peranemata. The same kind of partial transformation has also been seen occasionally in the contents of the large Confervoid cells, as shown in Pl. I., fig. 6.

In regard to these observations on the eggs of Rotifers, it seems clear that, as in the case of the encysted Ciliates, the appearances are altogether opposed to the supposition that there has been an entry of organisms into such eggs, followed by a devouring of their contents and subsequent multiplication of the invading organisms. The substance of the Rotifer's egg is seen to change simultaneously throughout its whole mass, and it is this altered and single mass which subsequently divides in the most irregular manner, and gives rise to the spherical bodies that speedily develop into Amœbæ, Monads, or Peranemata. The different stages in the origin of these forms has been followed, though there are gaps in the changes next to be described.

In another kind of Diglena resting egg the contents become transformed into **Monad Cysts** very similar to those whose origin has been traced within the resting spores of Vaucheria and also within small encysted Euglenæ[1]. I have never seen an embryo Rotifer develop in one of these eggs, though I have examined many dozens of them. These particular eggs are rather smaller than those whose changes have been shown in Figs. 31-35, and they have also minute conical projections here and there from the inner side of the outer envelope, one of which is plainly seen in the upper part of Pl. IV., fig. 36, A. In all other respects the visible structure of the eggs seems to be really similar to the others. There is the same evenly granular contents devoid of any visible nucleus; the same faint bluish tint of the envelope; and the same scarcely visible concentric markings of this envelope. Hence I conclude that it is the resting egg of a Diglena, though probably of a different species.

There is a further remarkable peculiarity favouring the notion of specific distinctness, which is that the heterogenetic changes undergone by the two eggs are also distinctive. None of the transformations already described in the other resting egg have ever been found in this one, and *vice versâ*.

The earliest stages of the change into the Monad cysts have

[1] Similar to those represented by Cienkowski as occurring in *Pseudospora nitellarum*. *See* p. 15, Note, and p. 19

never been seen. The eggs found have either been normal, as in Fig. 36, A, or else division of the egg-mass has already taken place into 6-10 spherical bodies. The majority of them have been in the stages represented by Fig. 36, B, C—that is, the spherical bodies have either been masses of granules enclosed by a definite bounding layer, as in B, or these granular contents have been distinctly separated from the cyst wall and have undergone concentration into a central mass, as in C. In a small number of the eggs, fully-developed Monad cysts have been seen, as in D, in which the contents have lost their granular appearance, and have become converted into a still smaller central mass of homogeneous, highly refractive, protoplasm. In this particular specimen these bodies are distinctly smaller and more numerous than usual.

It will be seen that the stages of development of these Monad Cysts correspond exactly with those of the similar bodies seen in Vaucheria resting spores (Pl. II., figs. 17, 18), and in certain small Euglenæ (Fig. 21). Although I have kept different specimens of these Rotifer eggs containing the Monad cysts under observation, beneath cover glasses, for over a week, I have never been able to recognise any further development under such conditions. Similar Monad Cysts which I have found abundantly in Nitella and Vaucheria have remained quiescent week after week, and the central protoplasm has been only known to divide into minute Monads after the expiration of about three months.

(*c*) Transformation of the Contents of Ordinary Eggs of Diglena, and of Resting Eggs of Lepadella, into Primitive Multitubular Sporangia; and of Resting Eggs of two Species of Brachionus into Primitive Multilocular Sporangia.

Sporangia, as hitherto recognised, are formed only in connection with the pre-existing mycelium of some Fungus, or else direct from swarm-spores. They are derivative, therefore; but in the observations now to be described they have had no such origin. They are produced as primitive structures, by heterogenesis, and are wholly independent of any Mycelium, though they ultimately divide into different kinds of swarm-spores, like ordinary Sporangia.

So much then for what I mean by "primitive" sporangia—a designation which after much consideration has seemed to me the least objectionable of those that have suggested themselves. The first examples met with were of the *Multitubular* variety.

They were found in considerable numbers in a gathering of Euglenæ and Rotifers which had been contained in a beaker under a very small glass shade (with little air therefore) resting on a window ledge. At the expiration of seven days multitudes

of ordinary **Diglena** eggs were found in the Euglena scum, and very many of them were undergoing the various changes now to be described.

In Pl. IV., fig. 37, A, a healthy, finely granular egg with its single nuclear-like body is seen; B represents an early stage of the ordinary development of the egg; while C shows the appearance of an egg in which heterogenesis is commencing. The whole texture of the egg-mass is altered; its substance has become more refractive, and it is studded with spherical, fatty-looking particles of different sizes. In D a confused tubular mass is being developed, and something of the same kind is to be seen in E, the envelope of the egg being as yet intact in each of them. In F and G, however, the envelope has been pierced in many places by tubular projections, the contents of which in G may be seen to be breaking up into swarm-spores, similar to those which have been abundantly voided from H. The swarm-spores were minute, delicate, spherical or ovoid bodies having slow movements, which were either emitted singly or in groups of 4-16 within an almost invisible envelope which they gradually ruptured. The specimens represented in H were killed with a dilute iodine solution, which more or less altered their appearance, though the lack of definition of many of them is due in part to their being out of focus.

On another occasion I saw almost exactly similar changes occurring in a number of Diglena eggs after they had been shut up in a small earthenware pot for four days.

Some of the resting eggs of **Lepadella** have been seen developing into similar Multitubular Sporangia, though the series is not so complete as the last, the emission of swarm-spores not having been seen. These eggs were found with others of the same kind already referred to (Figs. 28, 29), some of which were developing in the normal manner, while the contents of others were being transformed into Amœbæ and Peranemata.

The earlier stages of this new transformation, such as are seen in Pl. IV., fig. 38, B, C, are very similar to the early changes shown in Fig. 29. In such cases the whole substance of the egg is equally changed throughout. It becomes darker and has fatty-looking particles of different sizes thickly sown throughout its whole mass, such as are not to be seen during the stages of normal development into an embryo Rotifer. In B the spines are unusually short and much more numerous than usual, but this specimen was found with the others and seems to be only a variety of the same egg. In C and D similar fatty-looking particles are to be seen, and there is evidence of the mass beginning to undergo some sort of division. This is fairly evident in D and E, and is very comparable with what is seen in the Diglena

egg (Fig. 37, E, F). The Lepadella resting egg, however, has a much firmer envelope, and, instead of the protrusion of many tubes of exit for the swarm-spores, only one is seen in this particular specimen.

In the other two cases in which "primitive" sporangia have been found, the nature or form of the sporangia has been distinctly different—they have been *Multilocular*, rather than Multitubular. In both cases the changes have been seen in the resting eggs of species of Brachionus.

The first changes to be referred to occurred in comparatively small **Brachion** resting eggs of some undetermined species, the general form of which is very similar to that of *B. Bakeri*, as represented in Pritchard's "Infusoria," Pl. xxxviii., fig. 10. They were met with under the following circumstances. A small, wide-mouthed bottle containing about three ounces of pond water in which were a few Euglenæ and many specimens of Cyclops, together with Rotifers, was left for about six weeks, indoors, under a large glass shade. At the expiration of that time, when it was again looked at, all the Euglenæ were gone; and, apart from specimens of Cyclops swimming about in the fluid, there was only a quantity of light flocculent sediment at the bottom of the bottle. Examination of this sediment revealed large numbers of the small Brachion resting egg with which we are now concerned, so that I have been able to examine nearly a hundred of them.

The outer envelope in all has been of a light yellowish brown colour, and covered all over with small dotted markings. It is much larger than the egg itself, so that a considerable space intervenes between it and one extremity of the egg. This outer envelope is also generally curved as in Pl. IV., fig. 39, A, B. In not one single specimen of all the eggs examined has the formation of an embryo Rotifer been seen. The largest number of these resting eggs have been in their ordinary condition such as may be seen represented in Fig. 39, A A much smaller number have been found with an appearance of comparatively clear spaces among the granules of the egg, as in B, and I am rather at a loss to say whether this change is to be regarded as a commencement of the ordinary development into a Rotifer, or whether it represents one of the early stages of heterogenetic change. No nucleus of any kind is to be made out in the majority of the eggs, those of the A type; and in the eggs in which the clear spaces occur there is not the slightest evidence of any segmentation of the yelk-mass. I am inclined to think, therefore, that the change seen in B may be the commencement of those which eventuate in the conversion of the whole egg-mass into a Multilocular Sporangium.

The intermediate changes leading on to the production of this sporangium I have not seen. They seem to have gone through their phases previous to the fifth or sixth week, as those that had been thus transformed (and they were very numerous) were found with the loculi empty, except for a few swarm-spores left here and there in one or other of them. Some of the eggs of the A and B type were very dark and obviously dead; and I am disposed to think that most of them were in a very similar condition, as, though they were examined from time to time during a fortnight, no changes, either homogenetic or heterogenetic, seemed to be taking place therein.

Some of the eggs showed a few large empty loculi, as in Fig. 39, C, where only three could be made out; others, as in D, were distinctly multilocular. In some of them delicate tubes through which the swarm-spores had been discharged were seen crossing the space between the outer envelope and the egg, as in E; in others one to six delicate spherical swarm-spores were found at rest in one or other of the loculi. In F, for instance, six were seen in a single loculus, and five of these are shown in the photograph. In this specimen, also, there is what I have seen only on one other occasion, that is, a number of fine mycelial threads interlacing in the space between the two envelopes of the egg—doubtless developed from one or more of the swarm-spores which had remained therein. The mycelial threads are plainly to be seen in the photograph with the aid of a pocket-lens, and are fairly well shown in the illustration.

The other transformations into multilocular "primitive" sporangia were more completely observed. They occurred in some large resting eggs of *Brachionus urceolaris* (?), which existed in company with resting eggs of Hydatina, together with some brownish scum, on the side of a bowl. A quantity of Euglenæ with Rotifers had been placed in this vessel three weeks previously, and the vessel had subsequently been kept under a bell jar, at first in the open air and afterwards within the house.

Some of the scum containing these eggs was scraped off the side of the vessel, and placed in a small glass beaker, which was covered with a glass cap and then put into a dark cupboard, whose temperature was pretty constant and about 65° F.

The eggs when removed from the side of the vessel had a thin crumpled outer envelope, plainly visible in some of them but not so distinct in others. They were very dark in colour, and opaque, except for a number of light, roundish spaces irregularly distributed through them, as in Fig. 40, A. These clear spaces were somewhat similar to those seen in some of the smaller Brachion eggs (Fig. 39, B), only in these large ones they existed in all the eggs seen. They may, of course, be nuclei, but here

as in the smaller eggs there was not the slightest evidence of any segmentation of the yelk substance.

These large Brachion and other eggs were examined again after they had been in the dark cupboard for two and a half days. The resting eggs of Hydatina seemed to have undergone no change, but more than half of the Brachion eggs had lost their previous very opaque appearance, and also the nuclear-like bodies within them. They had become much paler and much more transparent. In some of them the yelk substance was still entire as in Fig 40, B (though it seemed now to be made up of minute corpuscles rather than granules); but, in the greater number of them, the egg-mass had become divided into large or small segments. One is seen in C where there is division into a few large segments in which developmental changes were obviously going on. One of the segments also showed a nuclear-like body in its interior, though nothing so distinct as this was to be seen in either of the other segments. This specimen was left under the cover glass, with the addition of distilled water, and placed on the mantel-piece under a large watch glass. Twelve hours later it was examined again when some of the segments were seen to be empty loculi, and lying outside were a number of remarkable swarm-spores which had come to rest (Fig. 41, D). They nearly all had a single, long, coarse flagellum, though the upper one in the figure may be seen to possess two. Other loculi were not empty, but were in different stages of development, some of them distinctly showing swarm-spores within. The appearance of this egg was almost exactly like the smaller one shown in Fig. 41, A, in which the large loculus to the left was quite empty. This latter egg when first seen, fourteen hours previously, was in just the same stage as Fig. 40, C. In Fig. 41, B, all of the loculi were empty, and the loose outer envelope of the egg was plainly seen. Distinct tubes of exit for the swarm-spores were very rarely seen in these eggs.

In a few of the eggs, however, the transformation was of a different kind. Their contents had divided into a very large number (several hundred) minute loculi, and through the envelopes of these particular eggs there were many tubes of exit, as may be seen in Fig. 41, C. In this egg also the loose outer envelope is to be seen. The swarm spores from eggs which had undergone this latter change were not seen.

These transformations were so remarkable that I collected from the side of the vessel all the other of these large Brachion eggs that I could find, and subjected them to exactly similar conditions. After having remained in the dark cupboard, in a small covered glass vessel, for two and a half days, a large proportion of this new lot of eggs was again found to have

undergone tranformation into Mutilocular Sporangia, as above described.

The changes in these four cases have been generically similar. In none of them has anything like Mycelium been seen within the eggs, except in the two small Brachion eggs after the formation and partial discharge of the swarm-spores, some of which by developing had doubtless given rise to it. The transformation in the space of two and a half days of the whole mass of the great Brachion eggs was most remarkable, and not of a kind, as I submit, which could be accounted for on the hypothesis of infection. Let it be supposed for a moment that the organisms found may be classed with the *Chytridieæ.* It may be said, as de Barry tells us,[1] that the *Olpidieæ* and the *Synchitrieæ* have no Mycelium, that their swarm-spores have single flagella, and that they are so far in accordance with what I have above described. But with these organisms the Sporangium is formed by the development of a swarm-spore that forces itself into the interior of the organism which it attacks. How little the appearances seen in Figs. 37 and 40, where all the stages of change have been met with, can be considered to correspond with the penetration of one or more swarm-spores and their development into Sporangia within the egg, each reader must be left to decide for himself. For my own part, after the most careful examination of very many of the objects themselves, I have been able to find nothing in support of such an interpretation. What I have seen has not been the growth and development of adventitious bodies within the eggs, but a change occurring simultaneously throughout the whole substance of the egg-mass, and its final transformation into these multitubular or multilocular structures, of which I have spoken as "primitive" Sporangia.

(*d*) Transformation of the Eggs of Hydatina into Ciliated Infusoria.

One of the last observations on the subject of Heterogenesis made by me in 1872, and the very last of which a pencil sketch was made in my note-book at that time, is thus referred to in "The Beginnings of Life" (Vol. II, p. 489): "The substance of some of the large thin-walled 'eggs' of *Hydatina senta* was seen to have undergone segmentation into about sixteen spheres, each $\frac{1}{1000}''$ in diameter. The external layers of these soon became condensed into cyst-walls, whilst the internal substance of each of them, after undergoing a series of molecular changes, resolved

[1] *Loc. cit.*, p. 166.

itself into an embyro *Oxytricha*, some of which might be seen revolving within their cysts. Some of this batch of Rotifers' 'eggs' were seen to be filled with such spherical masses, whilst others were observed in which a few of the embryos had escaped from their cysts, and were swimming about as well-marked specimens of *Oxytricha*, within the thin investing membrane of the Rotifer egg."

Unfortunately, nothing was stated, either in my note-book or in the book itself, as to the conditions to which these eggs had been subjected, and until early in the present year nothing of the kind was ever met with again. In the spring, however, I began to subject Hydatina eggs to different conditions in order to try to again bring about this remarkable transformation. After many unsuccessful attempts I at last found, early in the month of May, that the way to obtain this change was to place portions of Euglena pellicle containing the Hydatina eggs, or else masses of ten to twenty eggs carefully scraped with a scalpel from the side of a glass vessel on which they had been deposited, in some water in a small earthenware pot. The water was allowed nearly to fill the pot, over which the cover was placed, and it was then left undisturbed for three or four days at a temperature of about 65° F. This final attempt was made with a very small quantity of material, and unfortunately, owing to the previous setting in of dry weather, and the consequent drying up of all my sources of supply of Euglenæ, I was, much to my regret, unable to carry on the investigation.

Examination of the small amount of material enclosed in this pot, on the fourth day, was rewarded by the following discovery. I found a ruptured Hydatina egg, within which were three spheres exactly similar to what I had seen in 1872, which on measurement proved to be also $\frac{1}{1000}''$ in diameter; another similar sphere, whose contents had been converted into a small Ciliate, now actively revolving within its cyst; and with them a small free Oxytricha. The movements of these organisms were arrested with a dilute iodine solution in order that they might be photographed, but unfortunately this led to the dissolution of the free Oxytricha, so that in the figure (Pl. IV., fig. 42, B), it is represented only by a heap of fine granules just above the × marked thereon. The lighter of the three spheres, which did not stain so deeply with the iodine as the other two, is the one that contained the revolving embryo. The third motionless sphere was situated in a deeper plane, partly behind the one to the left of the figure. Close by the side of this ruptured egg there was another containing two similar spheres, and further away I found another ruptured egg membrane containing a single sphere of the same size and appearance, which, like the others, contained motionless contents. I had previously found

some remarkably altered eggs, which I thought might prove early stages of this kind of transformation. Fig. 43 represents two of these specimens, while a healthy Hydatina egg is shown in Fig. 42, A.

The summer being unusually dry it was not till after rain had fallen for some days that I at last succeeded, on September 22, in getting another supply of Euglenæ together with Hydatinæ. After placing them in water they were left undisturbed for a few days in order to obtain a good supply of eggs. Portions of the pellicle were then transferred exactly as before to small covered pots almost full of water, and they were then placed in a cupboard at a temperature of about 67° F. Examination of a portion of the pellicle at the end of the second day showed a large number of active and healthy specimens of Diglena; and a number of much altered young Hydatinæ whose development had progressed under these conditions, but not a single Ciliate of any kind. The Hydatinæ were imperfectly developed—mere shadows compared to what they should have been—and seemed to have taken no food. Examination of another portion of the pellicle at the end of the third day showed at once, to my surprise, a fairly large number of small Vorticellæ, all of about the same size. Very soon I came upon an unruptured Hydatina egg containing at least twenty spherical masses, slightly unequal in size (Fig. 42, C). Close by the side of this was a ruptured Hydatina egg, still containing five of the spheres (Fig. 42, D); while between the two eggs were three of the small active Vorticellæ. My suspicions that the Vorticellæ had developed from the spheres found in the Hydatina eggs was soon confirmed by finding spheres in which the embryos were more developed and about to emerge from their delicate cysts, as in Fig. 42, E. These embryos showed contractile vesicles, and in the larger of the two there was the usual differentiation of the oral and caudal extremities. Embryo Vorticellæ, unlike the young Oxytrichæ, do not revolve within their cysts, owing to the lack of cilia distributed over their surface. Several other eggs were found in the same and in subsequent specimens of this pellicle containing similar spheres; some of the eggs being entire, and others ruptured with part of the spheres discharged. In each specimen also there were a number of the Vorticellæ (but no other kind of Ciliate) such as I have succeeded in photographing (Fig. 42, F, G) after arresting their movements and slightly staining them, by means of a dilute solution of Westphal's 'mastzellen stain.' Other embryos were seen, just emerged from their delicate cysts, exhibiting their characteristic contractions of the posterior part of the body and the commencing formation of the pedicle, exactly as I had seen and

described many years previously.[1] The body of the developed Vorticella showed a very delicate transverse striation. Nowhere, however, could I find the early stages of this transformation of the Rotifer's egg into Ciliate matrices. Under the conditions to which these particular eggs were subjected, the complete transformation of many of them into Ciliates and their matrices had been brought about within three days.

The one set of Hydatina eggs gathered in the spring yielded embryo Oxytrichæ, while the other set gathered in the autumn, and exposed apparently to similar conditions, yielded Vorticellæ. Why should this have been? As to that I know nothing. Some very minute differences probably existed in what Herbert Spencer would term the 'physiological units' of the eggs. The facts, however, are so far harmonious with others previously recorded, tending to show that the forms of Ciliates are, to a certain extent, interchangeable (p 22, note 2).

It remained, however, more fully to study the conditions of origin, and to trace the early stages, of this remarkable transformation of the Rotifer's egg.

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Since the last sentence was written, a month ago, I have spent much of the leisure time at my disposal in studying the conditions under which these remarkable changes may be obtained. After very many trials, and not a few disappointments, I have been fairly successful, and am now able to indicate the means by which Ciliates may be obtained, not quite at will, but with a tolerable amount of certainty from Hydatina eggs.

Some failures may perhaps be due to the source from which the Hydatinæ have been derived. Thus, one of my early unsuccessful trials was due apparently to my experimenting with organisms obtained in water which had drained away, after rain, from a manure heap—the water itself having a distinctly brown colour. Since then I have always obtained the Rotifers, in association with Chlamydomonads and Euglenæ, from a ditch near Kingsbury into which there was a small amount of drainage from a farm yard, and where Hydatinæ have always been procurable in wet weather. Some of the surface mud, having a green scum on it, has been carried away, and as soon as possible put into a glass bowl into which about a pint of tap water was poured. The bowl was then placed on a wide window ledge, and covered with a large glass shade.

After twenty-four hours the water will be found to be pretty clear, and on examination with a pocket lens a number of Hydatinæ may be seen. There will, however, be an absence of

[1] "The Beginnings of Life," Vol. II., p. 464.

eggs on the sides of the glass. But after forty-eight hours, with temperature of 55-60°F, the Hydatinæ may be seen to be most numerous, and many hundreds of their eggs may be found round the sides of the bowl, over a zone about one inch in depth just below the surface of the fluid. It is these eggs (which we may be sure are less than twenty-four hours old) that are the best to be used. I have gathered them in groups of twenty to thirty, generally mixed with Euglenæ or Chlamydomonads, by carefully scraping them from the sides of the glass vessel with a scalpel. The small mass is then dislodged from the edge of the scalpel, and allowed to drop into a little earthenware pot containing tap water. Some of the fragments thus gathered will sink and others will float. Again, colonies of eggs may be found on the surface of the fluid, which are easily taken up on the scalpel, and floated off on to the water in the pot. My present experience leads me to think that the changes into Ciliates take place rather more abundantly in those eggs that sink to the bottom of the pot, while those at the surface of the fluid yield a larger proportion which go on to the development of much altered Rotifers—that is, after the pot has been closed and exposed to a temperature of 60-70° F. for two to four days. Still this has not always been the case.

Unless the eggs have been selected in this way, so that they are all of them known to be less than a day old, there will sure to be found, in any chance collection, a large proportion of eggs that are already in various stages of development, and a corresponding smaller number of the kind specially required, that is, eggs in which development has not yet been initiated. The conditions to which they are exposed in the dark pots produce a remarkable alteration in these developing embryos; and the younger they are when the exposure commences the more profoundly are they altered. There is an excessive development in them of spherical or pear-like outgrowths into various parts of the body cavity, such as are scarcely at all to be seen in a healthy embryo. The embryos themselves are also generally paler, and freer from fatty-looking granules than those which develop under normal conditions, as may be seen in Pl. V., fig. 44, where A and B represent two stages in the development of healthy eggs, and B and C stages in the development of eggs taken from one of the closed pots. In the upper part of A a portion of the organism is blurred, owing to movements in these parts having occurred while the photograph was being taken; and in B a normal embryo is represented, almost ready to emerge from its shell, and with its pharynx fully developed. In C there is the representation of an altered embryo in a very early stage of development, with lobulation beginning all through its substance; and in D we have an embryo further advanced, and showing an extreme amount of this lobulation. In each of these embryos there was a faint

brownish patch (more distinct in D) representing the developing pharynx. The lobulation seems to be produced partly by the overgrowth of a number of small pyriform glands lining the rotatory organ, and of others on the under surface of the pharynx; and partly by an overgrowth of ovarian tissue. The latter has often seemed to me to be distinctly in excess. The glands in the two situations above referred to were fairly distinct in Fig. 45—even though it represents one of the best of the shadowy embryos born in the dark pot. It had also taken some food and had thus become a little more substantial than when it was born.

Another point is of great importance, and also of great uncertainty, and that is, the time that should be allowed in each experiment before the pot is opened. During the month of October the temperature of the cupboard in which I have been keeping the small pots has varied between 67° F. and 58° F., and that has possibly given rise to some difficulty. I have discovered, much to my surprise, that the opening of the pot, and the exposing of its contents for a few minutes even, either to daylight or to gaslight, puts a stop to heterogenetic changes which had previously been going on rapidly. This has occurred on four several occasions; subsequent examinations at intervals of twelve and twenty-four hours showing no further changes in the eggs remaining in the pots that had been thus opened.

My experience hitherto as to the actual time required has been rather contradictory. In all cases, it is true, the transformations have occurred within four days. But apart from this, great uncertainty exists as to the minimum periods in which the changes have taken place. I am also not at all sure whether the rapidity of the transformation is much influenced by differences in temperature within the range above mentioned. Thus, even with a low temperature of 58-60° F., I have found numbers of Vorticellæ developed from the Hydatina eggs, and the latter partially empty, in the short space of thirty-five hours.[1] The development into small or large spheres (an intermediate stage presently to be described) may not be found, however, till forty-seven, or even seventy-two, hours have elapsed; though on another occasion they were most typically developed in thirty-three hours.

The safest course to pursue in future, therefore, would be to put a quantity of the same batch of fresh eggs into each of three pots placed under similar conditions, and to open them at

[1] It should be borne in mind that the homogenetic development of the Hydatina embryo takes place very rapidly. According to Prichard (*loc. cit.*, p. 446): "Ehrenberg stated that in *Hydatina senta*, eleven hours after the deposition of a complete ovum, vibration of the anterior cilia was visible, and in twenty-four hours the young being escaped from its shell." Development, however, does not always begin so soon.

different periods. For this plan large supplies of eggs would be needful.

In Fig. 46 there are shown different stages of the heterogenetic transformation of Hydatina eggs which had been removed from a dark pot after they had been exposed to a temperature of 63° F. for thirty-three hours. A and B represent early stages in the formation of a multitude of small spheres from the substance of eggs in which homogenetic development had not commenced—eggs which, when placed in the pot, had been evenly granular, as in Fig. 42, A. In C and D further differentiation has occurred, and the eggs have been converted into masses of small hyaline spheres having granular contents—D showing an egg thus changed, the surface of which had been purposely focussed. Fig. 47, A, B, show other of these eggs more highly magnified. In A the egg mass has undergone some shrinking (it had been kept for a time under a cover-glass), and the spheres are still in process of formation; while in *B* the spheres are fully formed and distinct, though varying much in size.

Large numbers of the eggs were in these particular stages of change, but this was one of the occasions in which development was stopped by the opening of the pot—though perhaps not completely so, as on a subsequent examination, twenty-four hours later, I found the egg represented in C, in which there were, among its other contents, two large and three or four smaller, motionless spheres, each having a vacuole in its interior. Two other eggs were partly empty, but each of them contained 15 to 20 young, active specimens of the ciliate known as *Aspidisca costata*. These eggs were partly covered by foreign matter, and, not being favourably situated, were not photographed; and I have met with no other similar specimens since.

On another occasion a pot was opened at the end of the second day, and very many eggs were again found in the stages of hetero genetic development above described and represented in Fig. 46. Others of them contained much modified embryos, and there were also many free, shadowy young Rotifers. Here also the changes were arrested by the opening of the pot. It will be observed that these changes are precisely similar to those represented in Fig. 43, which show some of the early stages of transformation met with last spring, when the matrices found within some Hydatina eggs developed into Oxytrichæ rather than into Vorticellæ. I have lately seen another Hydatina egg which contained ten similar motionless matrices and one small active Oxytricha. The motionless matrices which yield these dissimilar products are quite indistinguishable from one another, although a little later on, in the case of Oxytrichæ, the embryos begin to

revolve within their delicate cysts, and thus clearly show that we have not to do with young Vorticellæ.

In the first experiment that was purposely made with fresh eggs (all less than twenty-four hours old) the pot was opened after an exposure of forty-seven hours to a temperature of 60-63° F. These eggs were found to show a somewhat different kind of change, terminating in the formation of distinctly larger spheres, some of which were of just the same size as the more developed matrices that have been seen to unfold into Oxytrichæ or Vorticellæ. In Fig. 49, A, B, early stages in the formation of these spheres from the egg-mass are shown; while C and D represent many of the spheres much more completely formed, though varying a good deal in size. Large numbers of these eggs were seen undergoing the same kind of change, a portion of one of these masses being represented in Fig. 49. The comparative universality of the changes in this experiment showed the benefit of using the perfectly fresh eggs, but even the most cautious and brief exposure by removal of the cover and the abstraction of two portions for examination again checked all further advance.

The stages between those represented in Fig. 48, D, and in Fig. 42, C, have not yet been met with. But seeing that both of these changes have been many times found within unbroken Hydatina eggs, there can be no reasonable doubt that Fig. 42, C, represents a later stage of development of the products directly traced from the egg substance in Fig. 48. The spheres are about equally numerous in the two cases, and they are in each case also rather unequal in size. This inequality is even more markedly seen in Fig. 50, A, than it is in Fig. 42, C.

Then again there can be no doubt that the spheres which have during the last month been produced from the Hydatina eggs develop not into Oxytrichæ but into Vorticellæ.[1] Over and over again, in the specimens examined, there has been the common association of these young Vorticellæ with the unbroken or broken eggs of the Hydatina which have been removed from the dark pots. A group of such Vorticellæ around three egg shells is represented in Fig. 50, B, and in one of the shells an undeveloped matrix is still to be seen. The stages of the development of Vorticellæ from these matrices have also been traced. Fig. 42, E, shows the young organisms, having contractile vesicles, and about to emerge from their own delicate cysts; while multitudes of them have been seen with pedicles in different stages of development, some of them shorter than

[1] Only one exception to this has recently been met with (see p. 50).

that represented in Fig. 50, C, and others with pedicles fully developed as in Fig. 50, B, and Fig. 42, F, G. The fully developed form with expanded cilia has been most happily preserved in Fig. 50, D, with the aid of a dilute solution of Westphal's stain. It has very delicate tranverse striæ, and in many of them a slightly curved sausage-shaped nucleus has been made out. These organisms have been often seen undergoing longitudinal fission, the whole process occupying about three-quarters of an hour. The products are sometimes very unequal in size. One portion remains in possession of the pedicle, and the portion which is to separate, for some time before this is accomplished, develops a circlet of cilia near its posterior extremity, by the movements of which it at last twists itself away.

Not a few of the Hydatina eggs removed from the closed pots have been seen to be filled by a fine Fungoid mycelium, and a few of them have been found to become resolved into hundreds of delicate Monads.[1] The change which these latter eggs undergo contrasts very notably with those in which Vorticella matrices are developing, as may be seen by Fig. 51, in which A represents an egg of the latter type, and B and C others in which Monads are forming. The peripheral portions of the egg become comparatively translucent, and may be seen to be converted into what appears to be a congeries of very minute hyaline vesicles, each containing one or more granules; while the central portions still remain dark and merely granular (Fig. 51, B). In other eggs this peripheral portion may be seen to have resolved itself, in part, into a swarm of minute, active Monads. This was the state of things in Fig. 51, C, in which the upper and left portion of the translucent matter was still unresolved, while in the centre were a number of unequal, spherical or ovoidal, granular masses of uncertain nature. I tried to check the movements of the Monads with a 1 per cent. solution of formalin, and immediately that they were at rest proceeded to photograph the egg. At the expiration of the two and a half minutes needful for this process, being very doubtful as to what the state of the specimen would be, I took the opportunity of examining it, and found that the formalin had by this time almost dissolved the delicate Monads. This accounts for the hazy appearance of the lower half of the figure, which was originally filled by these minute Monads, together with four larger specimens, each of which had about five times the average bulk of the others.

Now comes the question as to the ultimate fate of the contents of those eggs which are converted into a mass of small spheres,

[1] This was also seen in 1872 (see "Beginnings of Life," vol. ii., p. 490).

such as are represented in Fig. 46. It is quite clear that they are not, as I at first thought might be the case, only early stages of the matrices that ultimately develop into Oxytrichæ or Vorticellæ. Fig. 48 shows the mode of origin of these latter matrices, and it is clear that they do not originate from growth or fusion of the small spheres. As I have already stated (p. 50), on two occasions, mixed with the eggs that have given rise to the small spheres, I have seen partially empty eggs containing 15-20 specimens of the small Ciliate known as *Aspidisca costata*. I think it highly probable that these small Ciliates may have been products of these eggs, though I have no absolute proof of it. Still in all the examinations that I have made during this month I have only come across the specimens of Aspidisca that were within these two eggs, and a few others in their immediate neighbourhood.

During the last ten days, however, I have frequently seen among the Rotifer eggs taken from the dark pots (even when eggs less than twenty-four hours old had been put into them) a number of the very large Ciliate known as *Otostoma Carteri.*[1] The mature specimens are very characteristic by reason of the ear-like shape of the mouth, their two contractile vesicles, and their very rapid movements of translation, associated with rotations of the body. At first I was very much puzzled to account for the presence of these large ciliates, many of which were found to be encysted, and though partly obscured by surrounding eggs were seen also to have undergone fission within their cysts into two or four segments which were in very active movement At other times such Ciliates were seen just out of their cysts, but still surrounded by Rotifer eggs or egg-shells only. Such a specimen, recently divided, is represented in Fig. 52, c, whose movements were stopped by a formalin solution.

At last I found some undivided specimens isolated, and revolving within their cysts; and careful examination made it clear that the cysts were really the shells of Rotifer's eggs. The whole mass of each was not only just the size of the Rotifer's egg, but the thickness of the membrane in each case was the same, and careful examination of the periphery revealed exactly the same appearance of very delicate concentric striæ in each case. Inside the egg-shell the embryo is contained within an extremely thin, diaphanous, and scarcely visible membrane. One of the first of these isolated eggs seen, which contained a single revolving Ciliate, was treated with a weak iodine solution, and in about a minute the movements of the embryo became so violent that I was afraid it would break through its cyst. I therefore ran some of a 1 per cent. formalin solution under the cover-glass as quickly as possible, and took it away to photograph it. On examination I

[1] See Saville Kent's "Manual of the Infusoria," p. 100.

found its movements were almost stopped, but the egg-shell was ruptured, and the embryo was moving out with extreme slowness. I could only take a photograph at 100 diameters, therefore, with an exposure of seventy seconds. The result is seen in Fig. 52, B, which also shows a ruptured egg-shell above. Subsequently the application of iodine caused another of these revolving embryos to rupture its cyst and come out; and as soon as it had come to rest the photograph was taken of which Fig. 52, A, is a copy. This was an immature specimen which had probably not long begun to revolve within its shell. Its resemblance to Fig. 46, D, or to Fig. 47, A, is very close, only there has been the development of short Cilia all over its surface. The specimens seen in Fig. 52, C, are older and therefore have become more organised; they had remained in their cysts without being disturbed till fission into two had taken place. The spherical bodies in their interior had diminished in number; their surface had become faintly striated longitudinally, and large contractile vesicles had developed in their interior. In the specimen on the left there is an indication of two vesicles, such as are commonly present in mature specimens of this particular Ciliate.

From what I have seen it seems to me clear that this is one kind of transformation that is apt to occur in the Hydatina eggs kept in small closed earthenware pots—but only in the case of that variety in which the egg mass resolves itself into a multitude of small spheres. The mass as a whole becomes converted into one large vigorous organism, just as in some of the large Confervoid cells, the whole contents of a cell become converted into a single large Amœba. In other cases the small spheres may individualise themselves and give rise to the small specimens of Aspidisca (though actual proof of this is wanting), just as the larger spheres become converted into matrices of Oxytrichæ or Vorticellæ. In all these cases the change is rapid and may be accomplished in two and a half days. I have found, as I have previously stated, the transformation of the eggs into small spheres in the short space of thirty-three hours, and I have recently found fully developed specimens of Otostoma in and among eggs which, in their fresh state, had only been put into the pot fifty-three hours previously.

Again, what has been shown by the photographs makes it superfluous to point out that the matrices of the other Ciliata found in the eggs of Hydatina have been formed directly from their granular protoplasm. The stages of the process have been pretty completely shown, whereby the substance of one of these eggs is converted into, and completely replaced by, 12-20 or more matrices which speedily develop into well-known forms of Ciliated Infusoria. The large Hydatina egg mass becomes

differentiated into large spheres, and the large spheres straightway unfold into higher and more complex organisms than we have had to deal with in previous sections. The whole process too occupies some period less than three days—and in many cases (where fresh eggs have been used) within five days after the Rotifer's egg has been laid it may, under changed conditions, give birth to its active brood of Ciliated Infusoria.

Clearly we have nothing to do with infection here, and, be it observed, in the numerous instances in which I have described the heterogenetic origin of organisms of lower grade—of Amœbæ, of Actinophrys, of Flagellate Monads, of Peranemata, or of Fungus Spores—the processes have been, in all cases, almost exactly similar. In the large Confervoid cells, in the resting spores of Vaucheria and Spirogyra, in the substance of encysted Ciliates, and in the eggs of smaller Rotifers, we have had to do in each case with a more or less simultaneous change throughout the whole of the parent mass leading to the separation of a number of spherical bodies, which subsequently unfolded into one or other of the lower forms of life above mentioned.

Some tentative Experiments as to Effects produced on Different Organisms or Eggs by cutting them off from Röntgen and Light Rays, or from Light Rays only.

As already mentioned, the transformations of the Hydatina eggs into Ciliated Infusoria that have been found this year have occurred when the eggs have been shut up in small earthenware pots almost full of water, and therefore with exposure only to a very small amount of air. They were purposely put into closed earthenware pots in order to cut off Röntgen rays as well as ordinary light rays. This was done after the cutting off of mere light rays, by placing them in a dark cupboard in small, covered, glass vessels, had proved ineffective. I had previously made many observations of both kinds with Euglenæ, and also with sprigs of Nitella, to some of which I shall have to refer on another occasion.

It is needless to repeat here what has been said in the last section as to the remarkable changes so rapidly brought about in Hydatina eggs, and their embryos, when they are simultaneously cut off from Röntgen and light rays, and as to the way in which such changes are immediately arrested when the access of light and Röntgen rays is permitted.

Similar comparative trials had been made with the large Brachion eggs to which reference has recently been made in section (*c*), leading to some very interesting results, as the following extracts from my note-book will show.

"Some of the large Brachion and 'resting' Hydatina eggs,

together with large encysted and slowly revolving Ciliates, were scraped off the sides of the vessel in the dead scum on which they were situated, and were divided into two sets: (*a*) being placed in a cupboard (temperature 65° F.) in a small earthenware pot; and (*b*) was placed in a small glass vessel, covered with a glass cap (so as to have no more air than in the other case), in the same dark cupboard—in order to cut its contents off from light but not from X-rays."

"On examination of the contents of (*a*), after two and a half days, it was found that the Ciliates had ceased to move and seemed dead, including large numbers of small Vorticellæ, and no changes had taken place in either of the Rotifer eggs in the way of development. They were probably not killed—though in regard to the Brachion eggs there was room for doubt as to this point Bacteria and Moulds had evidently very notably increased."

"Examination after a similar interval of (*b*) showed the large encysted Ciliates still revolving, and crowds of small active Vorticellæ (an increase). The Hydatina resting eggs were unchanged; but more than half of the Brachion eggs had lost their previous very opaque appearance (with large clear spaces or nuclei within). They had become much lighter in colour, and in many of them their contents were divided into large or small segments."[1]

"These observations have been repeated once since, with similar results in all respects as regards the death of the Ciliates in (*a*), and the transformation of the Brachion eggs into Sporangia yielding swarm spores as in (*b*), in the course of two or three days."

"It is notable that though the Moulds flourished in (*a*) they did not infect the Brachion eggs; while in (*b*), where Moulds do not seem to increase, these Brachion eggs become converted, in from two to three days, into many-chambered Sporangia."

My stock of these eggs was then exhausted, so that I could make no further trials. It is strange, however, that the particular Vorticellæ existing with these Brachion eggs should so speedily have been killed or rendered motionless by cutting them off from both Röntgen and light rays, while the ordinary Hydatina eggs, placed under similar conditions, should within a similar brief period have been transformed into other embryo Vorticellæ, which straightway developed and flourished.

I have since found that specimens of Diglena seemed to be comparatively unaffected after they had been cut off from Röntgen and light rays for three or four days. They were found as active as ever, taking food freely, and containing healthy-

[1] As shown in Fig. 40 and described on p. 43.

looking eggs—in fact, to have undergone no appreciable alteration, although under similar conditions some of the great Brachion resting eggs seemed to have been killed, and some of the ordinary Hydatina eggs had been transformed into different kinds of Ciliates.

Some observations of a comparative nature with fresh, healthy filaments of Spirogyra, bearing resting spores, are also interesting.

Some portions (*a*) which had been placed with water in a small closed earthenware pot were found at the expiration of three days to have their filaments for the most part dead and discoloured, though the resting spores remained unaltered.

Other portions of the same weed were placed at the same time in the same cupboard and into a similar pot, though instead of an earthenware cover a watch-glass only (*b*) was placed over it. At the expiration of three days (65-70 F.) these filaments were found to be green and healthy, and many had grown up out of the water, some of them spreading over the watch-glass.

At the expiration of the fourth day the differences above mentioned were still more marked. The weed in (*a*) was more completely discoloured, and dead throughout, though the resting spores were still scarcely at all changed; while in (*b*) the growth of the weed was remarkable. Its filaments were all over the sides of the pot above the level of the water, and all over the watch-glass. Still, notwithstanding this healthy growth, what appeared to be heterogenetic changes of different kinds were going on very freely in many of the filaments.

By the end of the sixth day, however, (*b*) was no longer flourishing. Very many of the filaments were found to be dead, and others were dying. The resting spores also had undergone some distinct alterations.

Thus this weed was killed within three days when Röntgen and light rays were cut off, while it actually flourished for four or five days when light rays only were cut off, and it was kept at the above-mentioned temperature in a vessel with a very limited air space. Perhaps if the small pot, instead of being covered over by a watch-glass, had been placed under a tumbler with more air, it might have flourished for a longer time.

Other organisms, such as Euglenæ and Nitella, are much less sensitive to the simultaneous cutting off of both Röntgen and light rays, and may remain distinctly longer under such conditions without undergoing any very appreciable change. The stability of their vital equilibrium is, however, far surpassed by that of many of the Confervaceæ.

As an instance of this I may state that, between six and seven weeks since, I scraped from the side of an aquarium a small

fragment of green scum, which was found to be composed of Oscillatoriæ, together with small Confervoid filaments and spherical cells of different sizes, containing bright emerald green, granular contents. Among these organisms were scattered a number of saline nodules which served to keep the organisms from being compressed by the cover-glass. The slip on which this specimen was contained, after a little additional water was added to the sides of the cover-glass, was then placed in a shallow, covered earthenware pot containing a thin stratum of water, so as to prevent the water beneath the cover-glass from evaporating too much. The pot was left on my work table, and the cover has been only removed about once a week for a brief examination of the specimen, and the addition of more distilled water to the edge of the cover-glass. I have just examined it again, and find, as on previous occasions, that the Confervoid cells are of as bright a green as at first; that some amount of growth has taken place; and that the great bulk of them show no appreciable change, although, with the exception of some very brief exposures, they have not only been beneath a cover-glass, but have been cut off from both Röntgen and light rays for nearly seven weeks.

These facts are very remarkable, especially when we bear in mind that confinement beneath a cover-glass alone, for a day or two, suffices to kill so many organisms. Under such conditions, too, heterogenetic changes (and especially the earlier stages) were in the great majority of cases arrested; so that the transformations taking place had to be studied, as is generally the case in embryological investigations, by the examination of different individuals in different stages of their several developmental processes. Still in a few cases, as I have shown, development, to some extent, did take place in the heterogenetic products of some vegetal and animal matrices under such conditions, so that in these exceptional cases I have been able to photograph the changes in the same specimen for two or three consecutive days. By this time, however, the products were generally killed, and there can be no doubt that the exposure for five or six minutes to the concentrated light from an oil lamp, needful for taking a photograph at 375 diameters, especially if the process were repeated, was in itself most harmful to them. I have seen, for instance, five or six Peranemata moving about within a cyst, and after examining them carefully for about ten minutes under a strong light, have seen them huddle together and become motionless, and the next day on examination, in their place, only a disintegrated granular mass has been found.

LIST OF ILLUSTRATIONS.

A mere list of the Illustrations is here given, together with the Enlargements in Diameters of the several objects represented.

For the description of the figures, the reader is referred to the text itself, at the pages indicated here, and on the plates after the numbers of the respective figures.[1]

PLATE I.

PLATE II.

[1] The essential portions of different Photographs have been cut out and placed side by side in groups, partly to diminish the expense of reproduction, and partly because they can thus be much more easily compared with one another. The plates are, therefore, really made up of 210 figures taken from separate Photomicrographs.

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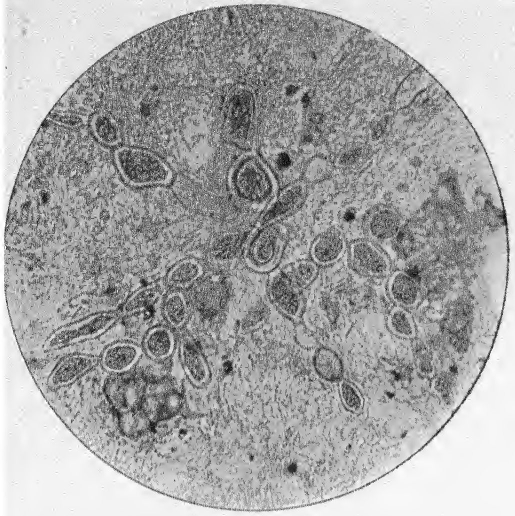

Fig. 1. p. 4.

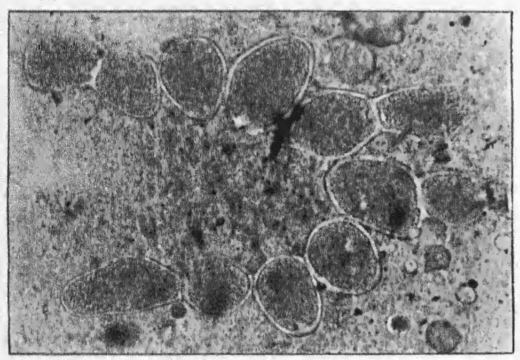

Fig. 2. p 4.

Fig. 3 p. 4.

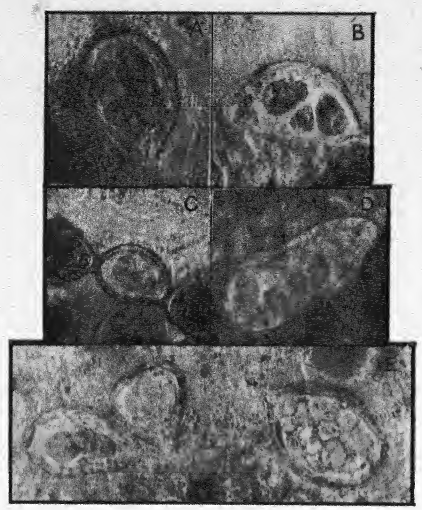

Fig. 4. p. 5.

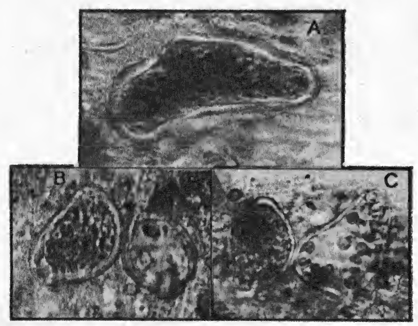

Fig. 5. p. 5.

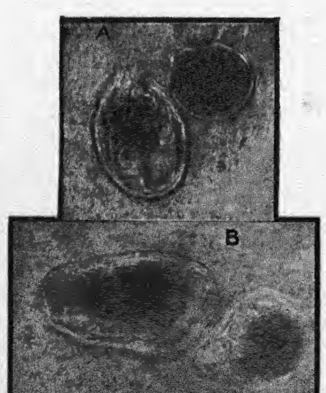

Fig. 6. p. 5.

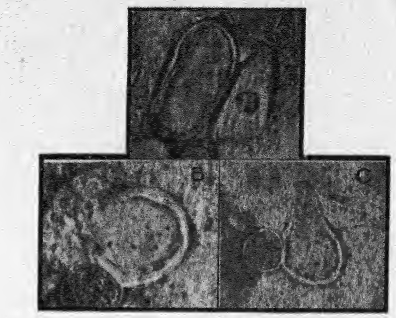

Fig. 7. p. 5.

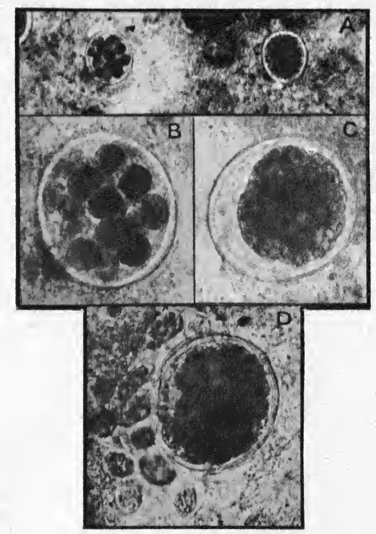

Fig. 8. p. 7.

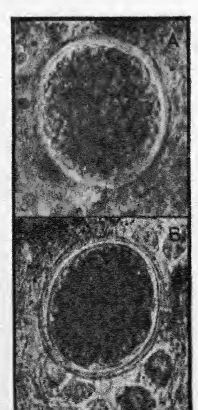

Fig. 9. p. 7.

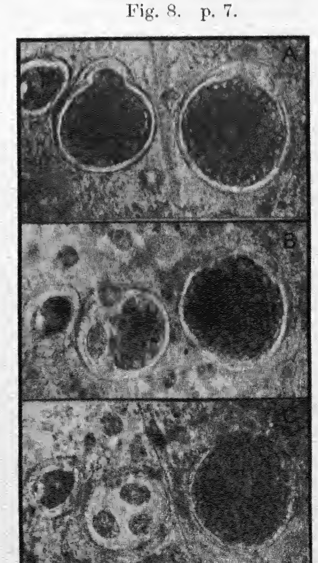

Fig. 10. p. 8.

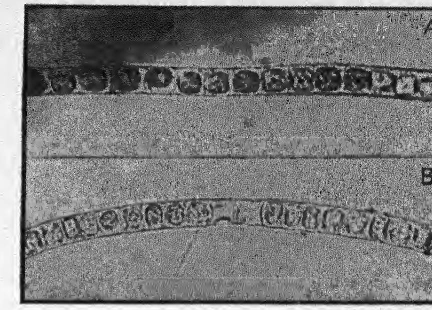

Fig. 11. p. 10.

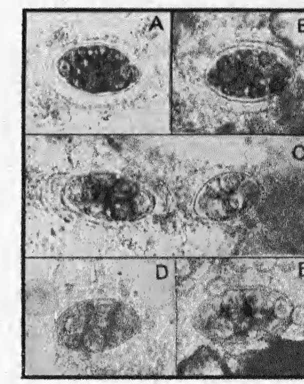

Fig. 12. p. 11.

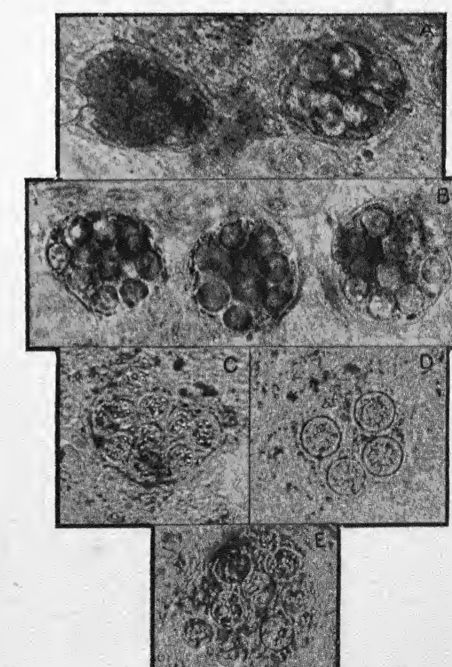

Fig. 13. p. 12.

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CONTENTS.

SECOND PART.

STUDIES IN HETEROGENESIS.

BY

H. CHARLTON BASTIAN, M.A., M.D., F.R.S.

SECOND PART.

IV. ON VARIOUS HETEROGENETIC CHANGES OCCURRING IN MASSES OF ZOOGLŒA GROWING IN AND FORMING PART OF THE PELLICLE WHICH FORMS ON THE SURFACE OF HAY AND OTHER ORGANIC INFUSIONS.

SOME of the changes to which reference is now to be made were briefly described by me in 1870,[1] and again more fully in 1872[2]. Although the changes then referred to were of a very remarkable nature, and were declared to be of such a kind as to lead to the production of flagellate Monads, of Amœbæ, and Fungus-germs from aggregates of Bacteria imbedded in a "more or less abundant, pellucid, gelatinous material," they seem to have attracted little serious attention, probably because they were so surprising as to be regarded as incredible. I am not aware of anything having been written since on the subject, either by way of confirmation or criticism.

Recently, however, I have devoted much time to a further study of these changes occurring in what has been named the "proligerous pellicle," and have been able not only to confirm the accuracy of the results previously recorded but also to considerably extend them.[3] The fact that the results of these fresh investigations will be illustrated from photomicrographs may perhaps cause more attention to be given to this subject in future

[1] *Nature*, No. 35, June 30, p. 172.

[2] *Proceed. of Royal Soc.*, 1872, vol. xx., p. 239, and "The Beginnings of Life," vol. ii., chap. xvii.

[3] At the time when my previous papers were published very little was known concerning *Zooglœa*; and that term was not employed in describing the constitution of the pellicle and of the "embryonal areas" occurring therein—though the latter were referred to as aggregates of Bacteria which had formed "around themselves a certain amount of pellucid, gelatinous matter."

by other workers. Certainly the field of observation is both interesting and extensive, and very much more work deserves to be done in this direction. My observations have, to a very large extent, been made upon the pellicles forming on the surface of infusions or macerations of hay, but much additional information would be derived from a wider study of the changes occurring in pellicles forming on other vegetal as well as animal infusions. A study of the pellicles forming in the former media will be likely to be much more fruitful than of those appearing on the surface of the latter, because animal infusions so speedily go on to actual putrefactions, which not only make their continued examination unpleasant but, as far as my observations have hitherto gone, tend in the majority of cases to limit the organisms found therein, apart from Bacteria, to Amœbæ.

Except when otherwise indicated, what I have now to say on this subject has reference to the pellicles forming on hay infusions. These have been made by macerating about one drachm of hay (cut into fragments of about an inch long) in five ounces of water, for periods varying from two to four hours and at temperatures between 75° and 120° F.[1] Of course the adoption of the longer periods and the higher temperatures, between the limits mentioned, will yield progressively stronger infusions—that is, infusions containing more and more of organic matter.

Experience has however gradually led me to the conviction that the various changes now to be described are to be made out better by using rather weak than strong infusions, because it is better that the scum to be examined should be comparatively thin. For a similar reason I have recently preferred to examine the pellicle on an infusion having a depth of one and a half to two inches, rather than one formed on an infusion having three or four times this depth—unless for certain reasons, to be hereafter mentioned, thickness of the pellicle is desired.

Of course hay is a substance which must necessarily present a considerable range of variation in every specimen from which an infusion is made. Differences will exist not only in the age of different specimens of hay, but also in regard to the kind and relative proportions of the different grasses and other plants of which it is composed. It will appear in the sequel also that notably different results will be obtained by the use of the same kind of hay in its different states and ages — that is with specimens taken (*a*) when growing but before the flowers have developed; (*b*) with others after the flowers have developed, but before the hay has been cut; (*c*) after the hay has been

[1] Thus for dry hay the proportion to water has been 1 : 40, but where fresh and moist plants or vegetables have been used they have been generally employed in the proportion of 1 : 20.

cut for two or three days only; and (*d*) after it has been stacked for various periods.

After the infusion has been made and allowed to stand for the requisite time, it has always been filtered through one, two, or three layers of German or Swedish filtering paper into a clean beaker, and subsequently covered with a cap of filtering paper, in order, while not cutting off air, to exclude excess of dust. In about twelve hours or so the fluid begins to show slight turbidity, and as this increases the light sherry-coloured fluid becomes gradually paler. In from twenty-four to thirty hours a thin almost invisible scum will have formed upon the surface of the infusion in the form of a coherent elastic membrane, and somewhere between thirty and forty hours multitudes of what I have termed "embryonal areas" of varying size and shape will have formed, and will be found to be imbedded in this thin scum or pellicle. When we attempt to remove a small portion of this early and almost invisible scum by means of a sterilised scalpel, we may often see that its point will very perceptibly depress the surface before it breaks through the membrane. Each day that passes adds to the thickness of the pellicle, till after seven or eight days have elapsed, if the infusion be not too shallow, it may have been converted into a comparatively thick pulpy layer, owing to continued accretions of fresh Bacteria from below, together with outgrowths projecting downwards. After this period, or even before, the superficial layer of the pellicle may gradually become more and more brown, while rounded or branched and elongated masses of zooglœa project still further from its under surface. Ultimately, after three or four weeks, the pellicle, if left, may break away and in part sink to the bottom of the vessel.

Such are the changes to be met with during the formation and growth of a pellicle on the surface of a hay infusion made and exposed in the manner I have described. One of the most notable points in connection with it is the fact that from a very early period the Bacteria which thus aggregate into a scum at the surface of the fluid, where they are freely exposed to air, excrete a transparent glœal substance by means of which the constituent units of this scum become blended into a thin elastic membranous layer.

As may be supposed, when we examine some of the turbid fluid, or the thin scum that first appears, Bacteria of several different kinds are pretty constantly met with; but, for the most part, with hay infusions, it is Bacilli which largely predominate, such as may be seen in Fig. 53, A. Toruloid corpuscles are decidedly rare, and no Monads, Amœbæ, or Ciliates are ever to be seen at this early stage. The mode and times of their appearance will be presently dealt with. In the first place,

however, I would emphasise the fact that the researches and the results which I am about to detail have no pretence to be conducted in ways that are proper and usual in the great bulk of Bacteriological enquiries. We start here confessedly with a mixed association of Bacteria, tending to aggregate in a promiscuous manner on the surface of the fluid, which soon pass into a resting stage, and then secrete a transparent glœal material that binds them together. The question with which we are now concerned is, *What are the microscopical changes apt to occur in such a scum or pellicle?* The scum is confessedly a composite zooglœal layer, and we are not concerned at present with the task of separating or identifying its constituent units. Our object will rather be to see in what way these units combine so as to produce new aggregates, small or large, which either singly or after many processes of fission, result in the production of several different kinds of organisms of higher type.

Before proceeding to answer this question in some detail, it is only right to point out that it is far from being the rule for all vegetal and animal infusions that the early organisms to appear are always Bacteria of different kinds. In some infusions made from carrot or from turnip I have found that minute Torulæ are numerous from the first. Then, again, it is not the rule for all infusions that the Bacteria which accumulate at the surface should begin at once to assume a zooglœal development, as is the case with those that reach the surface of hay infusions. On the contrary, in many cases the Bacteria remain separate, for some days at least, so that no coherent membrane is formed till a rather later period.

I entertain some doubts whether all the changes I am about to describe, whereby portions of the pellicle are converted, on the one hand, into multitudes of discrete corpuscles which subsequently develop into Amœbæ or Flagellate Monads, or, on the other hand, into myriads of Fungus-germs (some of which may be seen to develop into different kinds of Mould) should be regarded as heterogenetic changes or not. Certainly nothing in the way of relationship has hitherto been supposed to exist between Bacteria and the Monad-Amœba couple, or between Bacteria and Ciliated Infusoria; and though Bacteria have been held by some to have genetic relations with Fungi, this point of view has been almost completely rejected during recent years. Whether these latter changes, however, are in reality of heterogenetic type, or whether they reveal hitherto unconfirmed relationships—and that by a previously unsuspected nexus—they will prove equally novel to the great majority of biologists. In either case they would lie almost equally outside the pale of generally admitted facts.

(*a*) On the Production in and from the Pellicle of discrete Corpuscles which speedily develop either into Flagellate Monads or into Amœbæ.

At the expiration of three or four days, according as the temperature to which the infusion is being exposed is up to or some degrees below 70° F., the first indication of the formation of discrete corpuscles may be found. When a portion of the pellicle has been taken up on the tip of a sterilised scalpel, and rotated off on to a drop of water or of some staining fluid placed on a microscope slip, and when the cover glass has been applied, we find on examination a thin membranous, granular-looking layer surrounded by myriads of free and active Bacteria, though no free Monads or Amœbæ are to be seen. This layer will probably be already flecked by many of the embryonal areas, subsequently to be described, but on examination with a power of three or four hundred diameters some portions of the pellicle lying between them may be seen to display a more or less evenly granular appearance, owing to the Bacteria entering into its formation having in part ranged themselves at right angles to the surface of the fluid and being motionless, by reason of being imbedded in the viscid glœal material which they have excreted.[1] In many other places, however, especially when the pellicle is looked at slightly above the proper focal distance, appearances such as are represented in Pl. VI., fig. 53, B, C (× 500) are to be observed. That is, the pellicle in these situations is seen to be pretty closely packed with a number of minute, motionless, and rather ill-defined whitish corpuscles, which are always found to be located in the under layers of the pellicle.

I have over and over again noticed these appearances in the pellicle, and even more distinctly as in D (× 500) owing to the corpuscles being more fully formed, when there was not a single active Monad or Amœba in or around the portions under examination. But when other portions of the same pellicle have been examined twelve to sixteen hours later thousands of active Monads have been present, all of about the same size as the corpuscles previously seen, and each moving more or less rapidly by means of a single flagellum. These active specimens are to be found not only around the portion of the pellicle under examination, but beneath it where previously only motionless corpuscles existed (E, × 500).

For a long time owing to the discrete corpuscles in their motionless condition being situated on the under surface of the pellicle I found it very difficult thoroughly to satisfy myself as to

[1] Though motionless, they are far from being dead as Pouchet imagined. He says (*Hétérogénie*, 1859, p. 354): "La pellicule proligère étant constamment formée par les cadavres des animalcules dont les générations se sont succédé." He, in fact, invariably speaks of the Bacteria in the pellicle as "les cadavres."

their actual mode of origin. The general indications were certainly strongly in favour of their having been formed in and from the pellicle itself, as may be gathered from the following considerations :—

(1) Thousands of Monads appear comparatively suddenly of full size, and often when their appearance has been seen to be preceded by the presence of multitudes of motionless corpuscles of the same size in the deeper layers of the pellicle.

(2) Neither in their motionless condition nor in their free active state was there anything but very rare evidence of multiplication by fission occurring, although countless thousands of these Monads have been seen and examined from time to time.

(3) In the course of five or six days, when the weather is warm, Monads are in some pellicles formed in enormous numbers, and obviously at the expense of the pellicle itself, since whole regions of this membrane actually disappear where they have been formed, leaving only intervening ridges in the midst of which other motionless corpuscles appear to be forming. This condition of things is shown in Fig. 54, D, × 150, and also in Fig. 55, A, × 150—the latter illustration being from a specimen in which multitudes of Monads and Amœbæ, stained with logwood, are still seen between the ridges.

These facts and considerations alone, as I have said, seemed to indicate very forcibly that the corpuscles were formed from the very substance of the pellicle, and that they had not sprung up from multitudes of invisible germs, gradually growing till their full size had been obtained, and then multiplying with great rapidity.

After a time, however, I thoroughly satisfied myself that the corpuscles are, as a matter of fact, only individualised portions of the Zooglœa mass of which the pellicle is composed. I found it difficult to obtain photographs actually demonstrating this for some time, but I at last succeeded in taking one of which Fig. 54, A (× 375) is a copy, plainly showing a mass of these corpuscles originating in this manner, and each containing several Bacteria. Then, again, when the corpuscles separate and first begin to exhibit slow oscillating movements they may often be seen to have an exactly similar composition. They are then small pellucid spheres, having a single flagellum, and containing in their interior four or five Bacteria, exactly like those in the region of the pellicle from which they have been derived—one of them, though without the flagellum, being shown in B (× 500).[1] These Monads soon increase somewhat in size and become more active ; the Bacteria in their interior become dissolved and a

[1] Sometimes, however, the Bacteria are not so clearly recognisable, as I have above indicated, and the corpuscle as a whole is much more refractive. Similar differences exist also in regard to the segments into which embryonal areas divide.

distinct nucleus is formed. Some unusually large specimens from another hay infusion, in this fully developed condition, are shown in C (× 600). They were originally active and ovoid in shape, with a single flagellum, and when killed with a very dilute osmic acid solution presented the appearance seen in the figure.

Sometimes these discrete corpuscles rapidly develop into Monads, though at other times they may remain for days in a motionless condition; and in an old infusion where a certain amount of sediment exists this deposit may be found to contain multitudes of these corpuscles such as are seen in Fig. 55, B (× 500). Then, again, in an old pellicle zoogloeal projections of all shapes and sizes are apt to grow from its under surface, and in these projections after a time discrete corpuscles also begin to form in abundance, as may be seen in C (× 150). Specimens of these corpuscles more enlarged are shown in D (× 500). They were found in the projections from a pellicle three weeks old, and some of the corpuscles were seen developing into Monads.

In some pellicles the discrete corpuscles that are produced in the manner indicated develop into Amœbæ rather than into Monads. This probably depends upon some differences of an unknown nature in the chemical constituents of the infusion itself favourable to the manifestation of this phase of the Monad-Amœba couple. That the two forms are rapidly and easily convertible the one into the other is very generally admitted, and has also come much under my own observation.[1] In an infusion made from a bunch of *Melica nutans* in full flower Amœbæ were produced in myriads from the pellicles in this way. Fig. 56, A (× 375), shows a number of these Amœbæ varying much in size, which were found on the eighteenth day. Again, in an infusion made from mixed grasses in full flower (largely composed of *Lolium perenne*) a thin pellicle was observed, the weather being warm, in which Monads were scarce, but vast numbers of small active Amœbæ existed as early as the second day. In regard to the appearance of this pellicle on the fifth day my note-book says: "Three fourths of the pellicle is now apparently converted into Amœbæ, which exist in myriads. Not a single one of them has been seen to divide." In some ordinary hay infusions also Amœbæ are produced in great numbers from the discrete corpuscles. C (× 500) shows a number of small and large Amœbæ that were found with multitudes of others on the surface of a rather old pellicle in which they had had time to grow; while B (× 500) represents other Amœbæ beginning to encyst in another hay pellicle on the twelfth day, the upper one of which still exhibited very sluggish movements; and D (× 500) shows many others completely encysted, in which the protoplasm is, in some of them, more or less contracted.

[1] See *Proceed. of Royal Soc.*, 1872, vol. xx., p. 248, Fig. 3.

An infusion of roses made from the flowers, leaves and stems, or even from the petals only, often yields Amœbæ in great abundance. In the latter case the petals were allowed to macerate for about twelve hours before the infusion was filtered off. The Amœbæ found in such infusions are sometimes very short-lived. Thus, in one of these infusions they were very abundant and active on the fourth day, but two days later they were almost all found to have become encysted, and previous to this to have gathered together into such heaps as are shown in Fig. 57, A (× 250).

The production of Amœbæ in abundance is again practically invariable in mixtures of egg and water. When one tea-spoonful of mixed white and yolk of an egg is added to eight ounces of distilled water, enormous quantities of Amœbæ are to be found after a time in the pellicle which forms on such a mixture, though they have generally been preceded by the appearance of Monads. A very thick and glistening pellicle is commonly formed on such a mixture, and after six or seven days Amœbæ appear therein in great abundance. Their presence may be rendered most obvious by allowing portions of the pellicle to soak in a drop or two of logwood solution for some hours. Their actual mode of origin is apt to escape observation for long periods owing to the very thick pellicle in which they are formed. Still on a few occasions I have been fortunate enough to find very cogent evidence that they are formed in just the same way as the discrete corpuscles are produced from the pellicles on hay infusions—that is by individualisation and metamorphosis of minute portions of Zooglœa. Fig. 57, B (× 375), shows some Amœbæ originating in this way, on the seventh day, from the pellicle on an egg and water mixture. They were seen here forming from the very substance of the pellicle itself; while in the pellicle from another similar mixture, after four days (during part of which time it had been exposed to direct sunlight), vast multitudes of them sprang up close together in the manner shown in D (× 375). Some of these Amœbæ were still in the corpuscular stage, while others (almost completely motionless) had begun to assume irregular shapes. Three days later these Amœbæ, still small and about the size of a white blood corpuscle, had become very active. Teeming multitudes of them were found, though the mixture in which they were living had become extremely fœtid. Similar dense aggregations of embryo Amœbæ were found on another occasion on the twelfth day, though they were here a little further advanced—most of them showing sluggish movements. Such an aggregate stained with logwood is shown in Fig. 57, C (× 375). Soon such embryo Amœbæ begin to increase in size, and disperse themselves through the very soft Zooglœa of which the pellicle is composed. Such specimens, stained with

logwood, are shown in E (× 375). After a time, perhaps when unfavourable changes occur in the fluid, the Amœbæ begin to encyst themselves, as may be seen in F (× 375), representing a portion of a pellicle taken from an egg mixture on the twelfth day, in which multitudes of the Amœbæ had become encysted.

(*b*) On the Production from the Pellicle, as discrete units, of Fungus-germs which more or less speedily develop mycelial filaments therefrom.

While the discrete corpuscles which develop into Monads and Amœbæ are always produced from the under layers of the pellicle, those that develop into Fungus-germs, with which we are now concerned, are as invariably produced from the superficial layers of the pellicle. The fact of their origin from the substance of the pellicle itself is therefore much more readily made out than that of the discrete corpuscles with which we were concerned in the last section.

The discrete Fungus-germs may easily be seen to commence by the individualisation of small ovoidal or spherical portions of the pellicle, in which at first the contained individual bacteria are distinct as in Fig. 58, A (× 375), which shows them as they were originating on the third day on the surface of a hay pellicle; or in B (× 375) on the fifth day from a pellicle on an infusion of rye grass (Lolium); and again in C (× 375) from another infusion of the same grass made more than twelve months later.[1] In D (× 300) similar corpuscles taken from a pellicle on a carrot infusion on the third day are developing mycelial filaments.

It not unfrequently happens that the units which originate in this way become, as they develop, more refractive, as shown in the last two figures. They may also notably change in colour—becoming brown, or even brownish-black as they mature. This latter change is well seen in Fig. 59, A (× 375), which represents a portion of a pellicle taken on the seventh day from an infusion of hemp. At the periphery of the mass, below and to the right, numbers of ovoidal units are seen separating from the pellicle, and similar to it in colour. In others every gradation of tint may be recognised between these and the rather large brownish-black germs. Above, there is a lighter coloured patch, forming a sort of magma, in which germs are developing, as may be seen plainly in the photograph with the aid of a lens. The same kind of change in the colouration of the germs may be observed occurring in B (× 500), the material of which was taken from the pellicle of a hay infusion on the twelfth day.

[1] The grass having been kept in the meanwhile in a cardboard box.

Here the separate units as they developed became brown, and some of them are to be seen germinating into mycelial filaments.

Another curious and more obscure mode of origin of Fungus-germs has been made out in a hay pellicle three and a half days old, with the aid of a solution of magenta, which stained the separate units of a bright red colour. Their mode of origin and growth is shown in Fig. 60. They are seen in A (× 700) as very minute spheres or ovoids which tend to elongate and to undergo segmentation. In B (× 700) the same kind of units are seen but of larger size, and also at C (× 1,000). These germs commence as very minute units in the substance of the pellicle, whose ultimate origin cannot be traced. They seem, however, to grow rapidly, and to segment as they grow, so as to form irregular groups of ovoidal Fungus-germs.

Another mode of origin of Fungus-germs is sometimes exceedingly abundant in the pellicle on a hay infusion, forming a transition apparently between their mode of origin as discrete units and that which will subsequently be dealt with, where they appear as the result of the segmentation of embryonal areas. In this mode of origin small or large areas of the pellicle become altered, so that we have a dense packing together of minute units, forming a sort of magma, perhaps a shade or two darker in colour than the surrounding portion of the pellicle. Over this area, in minute foci here and there, a darker tint shows itself, owing to the appearance of mere specks or very short lines of a brownish colour, and these obscure markings (as the examination of contiguous more advanced foci seems to show) constitute the beginnings of a multitude of brown Fungus-germs, which gradually fashion themselves, and, as in the variety last described, rapidly grow in size as well as segment (Fig 61, A, × 700). In this way there are ultimately produced all over the pellicle large heaps of ovoidal light-brown Fungus-germs, each of which contains one, two or four minute spherical particles, as in B (× 500). Patches of this kind, sometimes so small as to yield only four or five germs, and at other times large enough to produce a hundred or more, together with all intermediate sizes, are extremely common in the pellicles on hay infusions, and sometimes may show themselves comparatively early. Thus in one specimen I recently noticed them fairly abundant on the fifth day, and twenty-four hours later (the temperature being about 70° F.) they had increased enormously, so that the pellicle had already assumed a slight brownish tint owing to the great abundance of small and large patches of these brown Fungus-germs. When incompletely developed small aggregates of these germs have a curious wrinkled appearance (due to indeterminate markings), but in their fully developed state the separate germs appear as ovoidal corpuscles such as are seen in Fig. 61, B.

(*c*) On the Production of Embryonal Areas in and from the Pellicle, and on the Development therefrom of Monads, Amœbæ, and Fungus-germs.

When portions of the pellicle on a hay infusion, prepared and exposed in the manner I have already indicated, are examined under a low power of the microscope, at periods varying from about thirty-six hours to three days after filtration, multitudes of whitish 'areas' varying greatly in size and shape may be seen covering the greater part of the surface under examination. These 'areas' are rendered specially distinct if the specimen destined for examination has been placed in a drop of a one per cent. eosine solution. This soon stains the Bacteria in the ground-work of the pellicle of a pale red colour, but leaves the 'areas' themselves unstained or rather of a bluish-white tint. Fig. 62, A (× 100), shows such a specimen taken from a hay infusion on the fourth day, in which the 'areas' were particularly numerous. B represents a portion of this pellicle similarly prepared but twice as much enlarged, showing a portion of one very large area and many minute ones. C (× 100) shows a number of very small areas from another hay pellicle, which had been exposed to a lower temperature for three days. This specimen had been stained with logwood, which colours the 'areas' of a deep red tint while leaving the Bacteria around almost unchanged. D (× 100) shows very minute 'areas' in an unstained portion of a pellicle taken after fifty-one hours from an infusion prepared from the petals of a tea-rose. The petals were allowed to macerate for seven hours at a temperature of 84° F., previous to filtration of the infusion through Swedish paper. It will be seen that the 'areas' are here so small as to be in part no larger than the discrete corpuscles previously described. They seem indeed to have yielded similar products, either singly or after segmentation, since two days later the infusion was swarming with myriads of Monads and Amœbæ.

Specimens subjected to a higher degree of magnification show well the constitution of these 'areas,' and make it plain that they are, in fact, in the first place (like the discrete corpuscles) only individualised portions of the pellicle. This fact is clearly shown by Fig. 63, A (× 375), representing a portion of a thin pellicle that formed on a mixture of white of egg and distilled water (1 : 3). Here small 'areas' are seen separating from the surrounding pellicle, and having obviously a similar constitution. B (× 300) represents other small 'areas' very similar to those shown in A, which were found in a portion of a pellicle formed on a hay infusion at the end of the third day, some of which have already segmented (apparently without undergoing much

further change) into amœboid corpuscles.[1] Two days later there was an enormous production of such amœboid corpuscles in this pellicle, which speedily developed into Monads. This transformation I was able to watch. Some of the motionless corpuscles after being exposed for a few minutes on the stage of the microscope to the light and heat from the lamp were seen, just as I described the change in 1872,[2] to develop a flagellum, and to become active Monads of an ovoidal shape.

This similarity in the constitution of the 'areas' in their early stages to that of the pellicle immediately around it will be further seen in many other specimens. C (× 500) represents a portion of a pellicle taken from a hay infusion after forty hours which was then immersed in a solution of logwood. An 'area' is shown in which segmentation is commencing, and the Bacteria entering into its composition seem precisely similar to those in the pellicle around it. This similarity between the constituents of the 'area' and of the pellicle around it is even more plainly seen in D (× 700), representing an 'area' found in a thin pellicle on another hay infusion after fifty hours, which is beginning to segment, and in which the Bacteria are larger than I have ever before or since seen entering into the composition of an embryonal area. This specimen had been for a few minutes in a weak magenta solution, and it will be found that the 'area' has taken up the stain unequally in different regions, while the 'area' shown in C has not taken up the logwood dye at all, or not more than the pellicle around—contrasting notably in this respect with a portion of another older and different kind of 'area' which is to be seen, on the left, by its side.

These differences in regard to the degree of staining are due to the varying chemical constitution of the 'areas' in their different stages of development. In the first stage, such as is seen in Fig. 63, A and B, we have to do with mere individualised portions of the pellicle in which the contained Bacteria are quite plain and distinct. At this stage the substance of the 'areas' has no more affinity for various dyes than is possessed by the pellicle immediately around. But at a later, or second, stage a distinct change of some kind in the constitution of the 'area' begins to take place Its substance grows more refractive, so that the Bacteria within are more or less obscured (the extent to which this occurs varying much in different kinds of 'areas'), and at the

[1] Such amœboid corpuscles, resulting from the segmentation of an embryonal area, seem to be essentially similar to what I have hitherto been speaking of as discrete corpuscles, and to be destined like them to develop either into Monads or Amœbæ. The discrete corpuscle originates separately; the amœboid corpuscle is derived from the segmentation of an 'area.' That seems the only difference.

[2] *Proceed. of Royal Soc.*, vol xx., p. 244.

same time the mass commences to segment more or less minutely, and in that way begins to show evidence of the potentialities gradually being acquired by this individualised portion of the pellicle. At this stage various dyes are taken up most freely. The chemical change on which this affinity depends becomes established in an irregular manner throughout the substance of an 'area.' Consequently when stains are used at an early stage in its development, we are apt to see a very irregular and patchy colouration such as is represented in D.[1] This gradual deepening in the intensity of the stain is also well seen in Fig. 63, E (× 375) which represents early as well as more fully developed stages of an 'area' of a different kind that was found on a hay pellicle after forty-eight hours, and stained with logwood.

The more and more minute segmentation of part of a large 'area' which has been stained with logwood is to be seen in Fig. 64, A (× 900). This illustration shows also one peculiarity to which I have already referred, distinguishing the transition from the first to the second stage in the composition of an embryonal area, since in the segments at the lower part of the figure the constituent Bacteria are still plainly to be seen, while in segments above these have become more or less indistinct. B (× 700) shows a later stage in the development of the same kind of 'area,' in which the process of segmentation has been carried still further. Sometimes, however, these stages of segmentation in which the products become progressively smaller are not seen. Instead of this a whole area may gradually change in its internal constitution, with only slight evidences of segmentation, and from it small ovoids or spheres may begin to separate here and there as in C (× 375). This same kind of change is seen taking place in D (× 500).

The formation of Fungus-germs from such ultimate segments of the 'areas' seems always to take place in a very similar fashion, though it is not always easy to make the process out. It is best seen in those cases such as are represented in Fig. 64, C, in which they are colourless and arise separately, or in only very small groups, from an 'area' that has undergone little segmentation. The steps of the process are these. We may start with an area such as is seen in Pl. vii., fig. 65, A (× 500), in which there are Bacteria of moderate size with no great abundance of glœa; and we may see in another of the same areas, B (× 700), lying side by side in the same pellicle, one of its latest phases in

[1] Further on we shall see that in many cases, under some conditions, such changes in the constitution of the 'area' are rendered obvious without the aid of any dyes, owing to the fact that the more mature regions of the area take on a brown colour—just as we have seen some of the Fungus-germs that originate as discrete colourless units grow brown, or brownish-black, as they become mature.

which segmentation has gone on into the bodies that are about to develop into Fungus-germs; but the actual production of the germs therefrom can only be made out in some cases where the matter of which they are composed is less refractive than usual. The process was recognised by me most clearly in one particular specimen in which the germs were forming singly or in very small groups. The Bacteria within the mass seem to increase in size in the terminal phases and to take on the form of simple ovoids, as may be seen in Fig. 65, C (× 850). But an examination of this same specimen shows that these ovoids appear as though they were nuclei in the centre of ovoidal masses of the glœa; and these nucleated ovoidal masses may also be seen separating from their matrix as so many distinct Fungus-germs. Sometimes, however, the area instead of giving birth to a number of single units, breaks up, as we have seen, into ovoids or spheres of different sizes, and these under favourable conditions may be seen to contain 2, 3, 4 or more of the ovoid Bacterial units, and ultimately to divide into just as many separate Fungus-germs.[1] It is curious how a little dye such as magenta completely alters the appearance of these Fungus-germs, as may be seen by D (× 850), which represents some specimens thus stained, though otherwise like those shown in C. When only lightly stained, the surface of the germs becomes so much coloured as to hide the nucleus; while if left in the stain long enough to enable it to penetrate to the nucleus, the envelope becomes so dark as to be completely opaque.

In the ultimate units of many other 'areas,' where the products have been smaller Fungus-germs, I have found that they have had a similar constitution, and have been composed apparently of one of the altered Bacterial units (of the zooglœal mass from which they have been derived) together with a certain amount of the original surrounding glœa—now metamorphosed into protoplasm.

Although these Fungus-germs are formed in amazing numbers on the surface of the pellicle as products of the segmentation of embryonal areas, they remain for the most part without undergoing further development. Still, occasionally, though not so frequently as when they have originated from the pellicle as discrete units, they may be seen to germinate and give rise to filaments of different kinds. Thus, in a hay pellicle on the sixth day I found the mycelium shown in Fig. 66, A (× 375) developing from a heap of spores, out of focus in the photograph and therefore only indistinctly seen below and to the right. In C (× 250)

[1] In the hay infusions, which I have dealt with of late, such Fungus-germs have been far more frequently met with as products of the 'areas,' than the amœboid corpuscles which develop into Monads or Amœbæ, though this has not always been the case.

another different kind of mycelium, but like the last of a blackish-brown colour, may be seen developing from a group of germs below, which as they mature exhibit dark outlines, owing to their becoming brown. This specimen, with some others of like kind, one of which is represented in B (× 200), was found in a hay pellicle on the eighteenth day.

In A the compartments of the mycelium are seen each to contain two spherical particles, while in the variety shown in B and C they generally contained four such particles. Some of the Fungus-germs which arise separately from a kind of magma as represented in Fig. 61, A (one of the commonest modes in which such germs arise), are shown in their mature state in B to possess from one to four particles in their interior, according as the original single particle remains undivided, or undergoes one or two processes of fission. The two kinds of incipient mycelia shown, therefore, in Fig. 66, may be only varieties developing from different states of these brown germs.

Different Varieties of Embryonal Areas.

Much variety is met with in the exact nature of the 'areas' encountered on different infusions, also in those in the same kind of infusion at different times, and even in the 'areas' that co-exist in different parts of the pellicle on the same infusion. A few of these variations, due to differences other than those of size and shape, which have already been fully demonstrated, I shall now proceed to illustrate.

On rare occasions a large 'area' like that shown in Fig. 67, A, (× 375) from a hay infusion, may be met with which has as yet undergone no segmentation. It is, however, the rule to find more or less of segmentation as in B (× 375). Then, again, 'areas' differ much as regards the amount or relative proportion of the glœa in which the Bacteria are embedded. This is rather more abundant in C (× 700) than in either of the other two, and still more plentiful in D (× 700), taken from another hay infusion on the fourth day, and having the ground-work of the pellicle stained with eosine. The amount of glœa reaches its maximum, however, so far as I have yet seen, in E (× 700), taken from a hay infusion on the fifth day, where the Bacteria are most unusually far apart. Similar areas are seen stained with thionine and beginning to segment in F (× 700).

The reverse condition has also been met with in another hay infusion in the course of the second day, that is, where the Bacteria are unusually closely packed, and there is comparatively little of the intervening glœa. These 'areas' also stained very slightly with logwood, as may be seen by Fig. 68, A and B, both the specimens from which these illustrations have been taken

having been immersed in this stain. A (× 375) shows an early stage of the 'area,' and B (× 250) a later one, in which segmentation is more advanced, and rather more of the dye has been taken up, though its substance has not yet become more refractive, and the contained Bacteria are still fairly distinct. This kind of 'area' has only been met with on a few occasions, and its later stages have not been traced. I have, however, seen almost exactly similar specimens in an infusion prepared (after a maceration for twenty-four hours) from the petals of a rose, and these segmented into spherical corpuscles which speedily developed into Amœbæ. An 'area' of this kind differs much, therefore, from one of the commonest kinds to be met with in a hay infusion, a specimen of which in the second stage is shown in C (× 700), where the substance has become highly refractive (the Bacteria being obscured) and segmentation is proceeding. A portion of this same pellicle that had been in a logwood solution for about a quarter of an hour is shown in D (× 700), in which a similar 'area' has taken up the dye most freely, while the pellicle around has remained unstained. On the right a patch of Zooglœa, in its first stage, may be seen, of the kind from which this area has been derived. In its last stage such an area resolves itself into Fungus-germs almost exactly of the kind shown in Fig. 65, C.

Other varieties of 'areas' are not unfrequently met with, in which the products of segmentation as they arise assume a brown or even yellowish colour. Such 'areas' sometimes occur at an early date, but they are especially apt to show themselves in old pellicles on hay infusions, the surface of which very commonly becomes of a brownish colour after seven or eight days. On one occasion I found 'areas' of this kind present in the pellicle on a hay infusion as soon as forty-eight hours after it had been prepared. These in their early stage were distinct 'areas' of an ordinary kind, such as may be seen in Fig. 69, A (500), near the centre of the figure. But, as may be seen more to the right of the figure, these 'areas' underwent segmentation, and from the segments there developed elongated groups of brown Fungus-germs. Then, again, in an infusion prepared from the common White Dutch Clover, after forty-eight hours small ordinary-looking 'areas' were formed (C, × 375), which gradually assumed a yellowish colour, and gave rise to a multitude of minute, rather elongated germs or groups of germs such as may be seen in the upper of the two 'areas.' Another example of this kind of change is shown in B (× 500), which represents one of several similar 'areas' found in a hay pellicle on the fourteenth day, after a portion of it had been slighly squeezed so as to render it less opaque. The area is commencing to segment, and at one extremity brown Fungus-germs are beginning to be formed therein,

which in other specimens I have seen separating from the matrix and becoming fully formed. A very similar sort of process has also been seen occurring abundantly in a hay infusion only five days old. Here there were a large number of 'areas,' some of them of very large size, more or less minutely segmented, but having a pellucid, highly refractive appearance. In these segments here and there brown masses were forming, having a curious wrinkled appearance, which underwent processes of fission, and thus split up into ovoidal Fungus-germs. In D (× 375), which shows only a small part of a very large 'area,' the brown wrinkled masses are seen, but not the ovoidal germs to which they give rise. Again, on the surface of a hay pellicle which had become almost uniformly brown by the eleventh day, I found quite one-third of its total area covered with heaps of brown Fungus-germs varying somewhat in size. After close and careful examination I satisfied myself that these germs developed in very much the same kind of way as those that are represented in Fig. 61, A. Only faint indications of definite areas could be made out, though in places without very distinct boundaries, the minute, closely-packed Bacteria assumed a brownish colour, gradually deepening in tint, and from these ill-defined aggregates were developed clusters of brown Fungus-germs, the individual units of which were sometimes small and sometimes fairly large, as in E (× 375). They seemed to be actually formed of different sizes in different clusters, and in these clusters to take origin separately, or at most in groups of twos and threes; subsequently dividing. This is an extremely common kind of change.

Some General Considerations concerning the foregoing Changes in the Pellicle.

The changes in the pellicle which have just been described are certainly remarkable, both in regard to their nature and their variety. What has been made out afresh during the last two or three years, and has afforded material for the photographs from which the illustrations have been taken, confirms in almost every respect the observations which I published in 1870 and in 1872, besides revealing many new facts. The strange thing is that, with the exception of the origin of Monads as discrete units from the pellicle, all the changes that have been hitherto dealt with were entirely unmentioned by Pouchet both in 1859[1] and in 1864[2]. He confined his attention almost solely to a question with which I shall presently have to deal, viz., the origin of Ciliates in and from the pellicle. And yet the development of

[1] *Hétérogénie.*
[2] *Nouvelles Expériences sur la Génération Spontanée.*

embryonal areas and the various other changes I have already described as occurring in the pellicle are infinitely more common and more obtrusive than any appearances connected with the origin of Ciliates. And, further, as I have already said, no subsequent workers, so far as I know, have published anything in regard either to the one or the other set of changes.

One of the most surprising things revealed by my investigations is the fact that the glœal material formed around Bacteria in those aggregations of them known as Zooglœa is often either incipient protoplasm, or at all events a material readily convertible into protoplasm.

In the early stage of an embryonal area we have to do with such a zooglœal aggregate, and within some hours, or at most a day or two, this aggregate undergoes a series of molecular changes in composition, and ultimately breaks up without remainder, at one time into a multitude of Amœboid Corpuscles, and at another into a multitude of Fungus-germs. The whole of the glœal material has by this time become converted into protoplasm, and in the case of the development of the Fungus-germs whose origin has been shown in Fig. 65, c, it is particularly clear that each altered Bacterial element carries off its own share of the previous glœal material, now transmuted into protoplasm.

Another point of much general interest is the fact that discrete Corpuscles (the progenitors of either Monads or Amœbæ), and also Fungus-germs, originate sometimes as separate units, and sometimes as ultimate segments of one of these embryonal areas—and that this difference in mode of origin does not seem to carry with it any appreciable difference in the respective products.

I cannot as yet say with any confidence from the mere appearance of an embryonal area, either in its primary or in its secondary stage, whether it will ultimately break up into Fungus-germs or into Amœboid Corpuscles. By far the largest proportion of those I have seen during my recent investigations have yielded Fungus-germs; but there is one kind of area, shown in Fig. 68, A and B, which I am strongly disposed to think is generally destined to yield Amœboid Corpuscles, as similar 'areas' did on the surface of a rose infusion. When working at this subject in 1872, with the particular specimens of hay then employed, I found I was able to determine with a considerable amount of certainty whether 'areas' should appear which would segment into Amœboid Corpuscles or not, by attention to the temperature at which the infusion was prepared.[1] Of late, however, with the specimens of hay used, there has not been anything like the same degree of certainty, though I have still reason to believe that such

[1] See *Proceed. of Royal Soc.*, 1872, vol. xx., p. 255.

units are least likely to appear when the infusions have been prepared with hot water maintained at temperatures much above 120° F.

As regards the mode of origin of Amœboid Corpuscles and Fungus-germs respectively as discrete units, the principle difference I have been able to make out has been the fact previously mentioned, that the former are produced from the deeper layers of the pellicle, while the latter seem always to originate in the superficial layer. Amœboid Corpuscles often originate as absolutely separate units, though just as frequently they spring up in larger or smaller groups, the units of which are almost in contact with one another. But Fungus-germs, when originating as discrete units, almost always spring up side by side in patches, either large or small, and are only very rarely seen as altogether separate units.

As I have already said, all gradations are apt to be met with between this origin of both kinds of elements by discrete units and by what I have termed embryonal areas, and in the one case as in the other it is plain, as I have shown, that in their origin we have to do with actual transformations of portions of the pellicle, small or great, into the new products.

Although the embryonal areas undoubtedly originate in this way by a differentiation of portions of the pellicle it is obvious that the portions of Zooglœa thus individualised continue to grow in bulk (sometimes to a considerable extent) and, from some altogether mysterious cause, to undergo more or less frequent processes of segmentation previous to their resolution into ultimate units of this or that kind.

The question, however, naturally arises as to whether this is the only way in which such embryonal areas may originate? And to this query I am able to reply, that it is not the only way. On two occasions I have been able distinctly to trace a commencement of another kind, in which the areas began with simple units, and continued to increase by growth and multiplication of the contained Bacteria and glœal material till large typical 'areas' were produced.

One of the observations to which I shall now refer was made upon a hay infusion prepared in the ordinary way, but at a low temperature, and which after filtration was exposed to the still lower temperature of about 58° F.[1] In this infusion, early on the third day, there was only a thin discontinuous scum, and in it I found numbers of a new kind of area in different stages of development. There were a number of Bacteria, single and dividing, surrounded by a large amount of glœa as in Fig. 70, A

[1] The other instance of this mode of development of embryonal areas was met with in a weak infusion prepared from dried Dutch Clover.

(× 700). There were others as in B (× 700), very lightly stained with carbo-fuchsine, in which division of the Bacteria into four and into eight had taken place; others as in C (× 700), in which growth with further division of the now more crowded Bacteria had taken place. All intermediate stages existed between these and large 'areas' like that shown in D (× 700), in which the Bacteria are still remarkably distinct, though the carbo-fuchsine which seems to have stained them has only in places tinted the glœa where it was beginning to undergo secondary changes. These areas ultimately segmented into Fungus-germs.

Another question of importance is why some masses of Zooglœa tend to individualise themselves and undergo processes of organisation and development such as I have described, while others seem to exhibit no such tendency. I have occasionally met in infusions of grass, though only rarely in hay infusions, very dense aggregates of Bacteria, looking like Zooglœa masses in which there is a minimum amount of the glœal material, that remain day after day without undergoing any change save some slight increase in size. Thus Fig. 71, A (× 500), shows masses of this kind as they were found in an infusion of grass on the twelfth day, in which Bacterial aggregates of the same kind had been seen from the second day onwards. Again, it is extremely common to find on the under surface of hay pellicles after three or four days, especially when the infusion is strong and the weather is warm, an enormous amount of Zooglœa forming an irregular though continuous stratum, in which there is an abundance of the glœal material, of the kind shown in B (× 375). This undoubted Zooglœal substance is so soft that the weight of a $\frac{3}{4}$ inch cover glass flattens it out into a thin stratum surrounding the portion of the pellicle to which it belongs. I have as yet seen no portions of it separate and undergo processes of organisation leading to the production of embryonal areas, though discrete corpuscles seem to be formed therein.

The reason of these differences is obscure, though in regard to the latter variety the absence of the formation of embryonal areas may be in part accounted for by the fact of its presence on the under rather than the upper surface of the pellicle, and consequently away from the direct influence of the air. As I have previously indicated, in a pellicle two or three weeks old extensive villous projections are apt to grow downwards from its under surface, and in these also embryonal areas are never found though discrete corpuscles spring up in abundance all through their substance, as well as other corpuscles, of which I shall have to speak more fully in the next section.

Although in the pellicle on a hay infusion there must necessarily be an admixture of many different kinds of Bacteria, the embryonal areas are probably in most cases formed from

aggregates of similar Bacteria—that is to say, 'areas' form in regions of the pellicle where similar Bacteria are massed together, now of one kind and now of another.[1] Upon slight differences in the nature of the Bacteria entering into the Zooglœal mass of which the 'areas' are composed, such differences as I have indicated in the appearances, mode of development and nature of the products from the several embroyonal areas may depend: though these respective characters of the different 'areas' may also be in part dependent upon the temperature and chemical conditions of the medium in which they are developing. The conditions must, of course, be liable to vary much in different infusions, looking to the varied proportions of the organisms existing therein, each of which, as a resultant of its vital activities, produces its own special changes in the medium in which it lives.

All this side of the question must remain for future elucidation.

I will only say here that I have recently had an opportunity of watching the changes in a thin pellicle, formed on an infusion of hay, which was composed almost solely of Micrococci rather than of Bacilli—a condition which I do not recollect to have ever seen previously. Yet portions of this pellicle isolated themselves in the ordinary way and 'areas' were formed therein which, though a little peculiar in the first stage (Fig. 71, C, × 500), subsequently gave rise to 'areas' having a very ordinary appearance, two of which are shown in D (× 500). These 'areas' ultimately, during the fourth and fifth days, segmented into amœboid corpuscles which speedily developed into Monads. By this time also an enormous amount of Monads had been formed from the pellicle as discrete corpuscles, while not a single Fungus-germ was as yet anywhere to be seen. Two or three days later, however, brown Fungus-germs began to appear in the greatest abundance all over the pellicle, partly as discrete units, and partly from the segmentation of small embryonal areas.

(*d*) On the Development of Ciliated Infusoria in and from the Pellicle which forms on Organic Infusions.

The observations now to be recorded have been made in the main upon infusions of hay, and upon mixtures of egg and water, though infusions prepared from plants other than those entering into the composition of hay have also been employed to a minor extent.

[1] This, however, is not invariably the case, as we shall see later on when considering the mode of origin of Vorticella Matrices (p. 108). Here there undoubtedly occurs in the first place an apparently promiscuous mixture of Spirilla and Bacilli.

It will be best first to consider the evidence I have been able to obtain from a study of (1) infusions of hay and other plants, and then (2) the evidence as yet derived from the examination of mixtures of egg and water.

(1) On the Appearance of Ciliates in Infusions prepared from Hay and other Plants.

If Ciliates are to appear in infusions of hay or other plants the infusions should be made by steeping the plants for two to four hours in water at a temperature of 70-80° F. The fluid should then be filtered into a clean beaker or other glass vessel through one or more layers of Swedish or German filtering paper. The vessel containing the filtered fluid may be placed under a bell-jar or have its top loosely covered with a cap of filtering paper in order to protect the surface from the advent of much dust.[1] The infusion may be either left exposed to ordinary daylight or placed in a dark cupboard without apparently influencing the result very much, so long as the temperature in the two situations remains about the same. The temperatures proving most favourable for varied changes in the infusions seem to be those lying between 60° and 75° F. Temperatures higher than this are, as I have found, less likely to lead to the production of Ciliates in the pellicles that form on most infusions.

It must not be supposed, however, that one has only to prepare an infusion of hay in the manner I have mentioned, with the result that after a variable number of days Ciliates will certainly appear therein. This is far from being the case; hay being, as I have already intimated, such a very variable mixture, both as to the different proportions of its various constituents and as to its age. As a consequence it sometimes happens that particular kinds of organisms will appear in vast numbers during the early days in some infusions, and the result may be the production of a state of things in the pellicle and in the subjacent fluid which is unfavourable to the evolution of Ciliates. Thus, on rare occasions, I have found spore-bearing Bacilli present and produced in enormous numbers in the pellicle during the first two or three days, and where this occurs ulterior developmental changes of any kind in this particular pellicle are apt to be arrested.[2] More

[1] When we deal with an infusion four or five inches in depth, in order to obtain a thick pellicle from which in the course of two to three weeks villous growth may form and be examined, it is well to diminish evaporation by allowing the bell-jar to dip into a shallow vessel containing water.

[2] This development of spore-bearing Bacilli I have found most prone to occur when an infusion has been prepared with hot water varying from 120° to 140° F., rather than with water somewhere between 70° and 80° F.

frequently, however, during the fourth or fifth day Monads or Amœbæ are produced in such enormous numbers from the pellicle that the chemical changes to which they give rise may prove harmful to the production of Ciliates from the small intervening regions of the pellicles which remain. The state of such a pellicle has been shown in Fig. 54, D, and Fig. 55, A. Certainly, on several occasions when, from previous trials with particular samples of hay, I had been in full expectation of finding Ciliates in the pellicles, they did not appear, and there was found instead an altogether unusually abundant production of Monads or Amœbæ.

Events of this kind, as well as differences in the mode of preparing the infusions, having led to many failures in my attempts to find Ciliates in the pellicle, I was induced to make some comparative observations which have led to the recognition of facts of much interest, bearing upon the relative productivity of infusions made from hay at different periods before and after it has been cut.

Some data in regard to these points obtained in the summer of 1900 were very surprising and unexpected. I therefore made other related observations in the summer of 1901, and these yielded essentially similar results, tending to show that the appearance of Ciliates in the infusions, as well as their great productivity in other directions, was likely to be ensured by making the infusions from thoroughly ripe or from recently-cut mature grasses, rather than from those which were still green and immature. This will be clearly brought out by a study of the subjoined abstract of observations made upon various infusions prepared from living grasses of different degrees of maturity, and from recently dead mature grasses, all the infusions having been prepared in the same way and exposed to similar conditions.

Observations made in 1900.

1. Rye Grass (*Lolium perenne*) *Flowers in bud.*
   2nd Day. Pellicle very thin. No 'areas.' No Monads.
   4th Day. Monads swarming. No 'areas.'
   9th Day. No essential change. Pellicle still very thin. Fluid almost clear. Still no 'areas.' No Ciliates.

2. False Oat Grass (*Arrhenatherum avenaceum*). *Flowers in bud.*
   2nd Day. Pellicle very thin. No 'areas.' No Monads.

Observations made in 1901.

1. Rye Grass.[1] *Flowers in bud.*
   4th Day. Pellicle thin. Monads scarce. No 'areas.'
   7th Day. Monads numerous, and some small Amœbæ. No 'areas.' No Ciliates. Pellicle still thin. Fluid almost clear.

2. Rye Grass. *Now in full Flower.*
   2nd Day. Pellicle thin. Monads abundant. No 'areas.' No Ciliate Matrices.[2]
   4th Day. Monads scarce. Small Amœbæ plentiful. No 'areas.' No Ciliate Matrices.

[1] This hay was composed in the main of Rye Grass, and all the specimens of it used this year were taken from the same part of the field.

[2] For the meaning of this term see p. 90.

Observations made in 1900.

4th Day. Monads swarming. No 'areas.'

9th Day. Pellicle fairly thick, and fluid still distinctly turbid. No 'areas.' No Ciliates.

3. Hay, *very over-ripe, but not cut; composed largely of Holcus lanatus.*[1]

2nd Day. Pellicle thick. No areas.' No Monads.

4th Day. Monads swarming. Matrices and free Kolpodæ very numerous.

9th day. Pellicle thick; brown on surface. Multitudes of Matrices and free Kolpodæ, large and small. Fluid still very turbid.

4. Hay. *Mixed grasses; cut three days.*

2nd Day. Pellicle rather thin. Monads swarming. Many Matrices of large Kolpodæ.

4th Day. Matrices and free Kolpodæ very numerous.

9th Day. Matrices still very numerous, though no free Ciliates seen. No mention of the existence of 'areas.' Fluid clear.

Observations made in 1901.

6th Day. Three-fourths of pellicle now replaced by small Amœbæ, myriads of which exist.

7th Day. Almost all Amœbæ now encysted.

3. Rye Grass. From same site, seven days later. *Grass now in seed, but still green.*

2nd Day. Pellicle moderately thick, swarming with Monads. No Amœbæ. No 'areas.' No Ciliate Matrices.

4th Day. Pellicle infiltrated with discrete corpuscles. No Amœbæ. No 'areas.' No Matrices.

7th Day. Now many active Amœbæ as well as Monads. No Matrices or Ciliates. No 'areas.' Fluid only slightly turbid.

4. Rye Grass. *Cut one day.*

2nd Day. Pellicle thin. Fluid very turbid. No 'areas,' Monads, Amœbæ, or Matrices of Ciliates.

4th Day. Monads swarming. Many Kolpoda Matrices and a few free Kolpodæ.

7th Day. Multitudes of discrete corpuscles forming, occupying about half the pellicle. Still many Matrices and some free Ciliates. A few 'areas.'

14th Day. Monads and small Kolpodæ still swarming. Pellicle thick. Fluid very turbid.

5. Rye Grass. *Cut seven days, taken from a 'hay cock.'*

2nd Day. Pellicle moderately thick. 'Areas' numerous.

3rd Day. Kolpoda Matrices, but no free Ciliates. No Monads. Many 'areas.'

4th Day. Great increase of Kolpoda Matrices, and many free Ciliates. No Monads.

5th Day. Myriads of Monads. Matrices and free Ciliates still very abundant.

A point of extreme importance brought out by these observations is the fact that Ciliates did not show themselves when

[1] In this and in each of the other cases—the grass having been cut by myself—only the upper half of the stem with flowers was taken.

infusions were prepared from living grasses, while they did speedily appear in infusions prepared from similar grasses when dead, or almost dead, as in the case No. 3 in 1900. The experiments Nos. 2 and 3 in 1901 seem to show some increase in the productivity of the infusions when they are prepared from grasses that have reached maturity; while Nos. 4 and 5 show a very distinct increase in their productivity when prepared from grasses that have been dead only a few days.

It is in infusions made from dead grasses also that embryonal areas appear; and, as far as my experience goes, these are to be met with in the pellicles on all infusions of hay, however old the hay may be. The same cannot be said, however, in regard to Ciliates. They may pretty certainly be present in infusions made from new hay, but with old specimens of hay the result seems to be more doubtful. The only definite statement I can make on this subject is that I have found Ciliates in abundance on some occasions when infusions were made from the same stock of hay that was used in No. 4 of 1900 up to eighteen months later, when the stock was exhausted; the hay in the interval having been kept in a cardboard box. On other occasions in which Ciliates have been found when old hay has been used I have had no certain knowledge in regard to the age of the several specimens.

The fact that Ciliates have not appeared when the infusions have been made from immature and living grasses, and that one kind of Ciliate, and one only, speedily appeared when the infusions were made from ripe and recently dead grasses, must be considered a fact of cardinal importance. It speaks against the notion of the appearance of the Ciliates in the infusion being due to the fact that the grasses are contaminated with such organisms either from the air or from others which had made their way up from the soil. If they had come from either of these sources they should have shown themselves in infusions made from living grasses just as certainly as in those made from dead grasses. It should be borne in mind also that each of the infusions above referred to had been passed through the finest Swedish filtering paper.

The Ciliated Infusoria which, so far as my observations go, have been met with in hay infusions, and, with few exceptions, in the other vegetal infusions with which I have experimented, have all been species of Kolpoda. The specimens vary, however, much in size, not only in different infusions, but also, to a lesser extent, in the same kind of infusion. Some of the largest matrices from which these Ciliates issue are to be met with in hay infusions, where I have often seen them as much as $\frac{1}{350}$ inch in diameter. On the other hand, about the smallest Kolpodæ I have ever found have been encountered in an infusion prepared from Yellow Bed Straw (*Galium verum*), where many of them

have not been more than $\frac{1}{2000}$ inch in diameter. They have been small also in infusions made from Dutch Clover (*Trifolium repens*), though not nearly so small as in the last-named infusion. Notwithstanding this great variation in size the Kolpodæ are in other respects very similar, being fairly rotund, somewhat reniform in shape, and covered with short cilia. Each organism has a single, large, spherical nucleus (not easily visible without the aid of stains), and a much more obvious large contractile vesicle at its posterior extremity.

These Ciliates make their first recognisable appearance in the substance of the pellicle, in considerable numbers, somewhere between the third and the eighth day. They appear as spherical encysted masses, having the appearance in their primary stage of being mere individualised portions of the substance of the pellicle itself, bounded by a delicate limiting membrane. Such bodies I shall continue to speak of as Ciliate *matrices*. The contents of each gradually becomes converted into an embryo, which may be seen rotating within the limiting membrane, now rather thicker and constituting a cyst. It may perhaps undergo fission once, twice, or thrice before the individual organism, or the products of fission, are enabled to free themselves from the enclosing cyst, and appear as active, free-swimming organisms.

These several statements, and other important facts concerning the origin and appearance of Ciliates in organic infusions, I shall now proceed to illustrate in detail.

Certain small matrices found on the eighth day on a hay infusion are seen in Fig. 72, A (250), to have the usual thin envelopes met with in the first set of matrices that appear in a hay pellicle. The irregular manner in which the matrices are found distributed over the surface of the pellicle is also shown in B (× 60), the material for which was taken from a rather old hay infusion, in which the matrices, as here seen, are apt to vary a good deal in size, and in the thickness of their envelopes. The substance of the matrices stains freely with various dyes—that is, much more freely than the surrounding substance of the pellicle, so that in their early stages they may with the aid of such dyes often be more easily recognised. The best stains to use for this purpose I have found to be Ehrlich's eosinophile fluid which, in many infusions, will stain the early matrices of an old-gold colour; or else Westphal's mastzellen stain. The solution should be very dilute—about 1 : 20 for the former, and 1 : 60 for the latter. Although these stains help to reveal the presence of the matrices in their early stages,[1] they very soon kill them, and they do not assist us in making out their

[1] So long as the fluids have a neutral reaction.

actual structure; on the contrary, they rather obscure it at times. So that, though I at first used them much, I have now come to the conclusion that unstained specimens are generally the best. But, as I have said, the stains named are often most useful in enabling us to detect the early stages of the matrices.

What may be made out as to their formation and ultimate composition is illustrated in part by Fig. 73, in which the different specimens represented have been obtained somewhere between the third and the fifth days from the pellicles on different infusions. In A (× 500), we have an aggregate of Bacteria, as an individualised portion of a hay pellicle, slightly stained with chrysoidin, and separating therefrom in the same kind of way that we have seen the discrete corpuscles and embryonal areas individualising themselves and separating from the pellicles in which they have been formed. In B (× 500), we have an unstained specimen which is obviously a mere spherical aggregate of Bacteria similar to those existing in the pellicle around, and as yet without a definite bounding membrane. A similar identity between the Bacterial contents of the matrices and those existing around them will also be seen in each of the other specimens represented in this and in the next figure. So that in this important respect the embryo matrices agree exactly with what we found to hold good for the embryonal areas. A matrix, stained with eosinophile solution, which was found in an infusion of *Melica nutans*, is represented in C (× 375), while in D (× 375), we have two spherical aggregations of Bacteria, the smaller of which is very distinctly developing a limiting envelope. In E (× 250), we have a small matrix found in a hay pellicle with many others, and staining exactly like them with mastzellen fluid. It is a mere aggregate of minute Bacteria though bounded by a distinct limiting membrane. In F (× 375), we have a larger matrix, unstained, and from a different infusion. Here the bounding membrane is very distinct, though there is as yet no sign of a nucleus within the enclosed mass.

This question as to the early composition and development of the matrices being so very important other illustrations are furnished in Fig. 74. In A (× 375), a nucleated matrix stained with mastzellen fluid is represented from a hay infusion. It is again a mere aggregate of Bacteria having a distinct limiting membrane. Two of these rudimentary matrices with clearly-defined limiting membranes, but no trace of a nucleus, from an infusion of *Melica*, are shown in B (× 375). The dark masses in this and in the next two figures are groups of brown Fungus-germs out of focus. C (× 250) shows a group of matrices in different stages of development from a hay infusion on the fifth day. The specimen from which this photograph was taken had been beneath a cover-glass for twenty-four hours previously,

in a very dilute solution of mastzellen stain. The pale vesicular bodies are mature matrices which had, in the meantime, become much altered by the formation of numerous vacuoles. Four immature matrices, however, are well seen. The one above and to the left represents one of the earliest stages of individualisation from the pellicle; while three rather more advanced specimens are situated close together to the right. The dark bodies obscuring them are, as I have said, brown Fungus-germs out of focus, but in the two upper specimens nuclei in process of formation are to be seen. D (× 500) shows another of these bodies, more enlarged, from the same specimen, in which development has advanced rather further, the nucleus being now very distinct, and having taken up more of the very dilute stain than the body of the matrix, and this again rather more than the surrounding pellicle. The similarity in composition between the substance of the matrix and that of the pellicle around is well seen here as in the other specimens, and actual examination under the microscope showed that the matrix had the same composition all through its substance.

Sometimes the matrices that form in this way are very large, as may be seen from Fig. 75 (× 375), showing three stages in the formation of what appear to be matrices of this kind from minute Bacteria. They were found in a hay infusion on the seventh day, in which multitudes of other matrices were also appearing. In A we have an aggregation still forming; in B one further advanced, with a limiting membrane commencing; and in C a nearly mature matrix of the same kind in which the Bacterial contents have aggregated in part into small spherical masses, and a contractile vesicle has formed, which is faintly shown on the right side of the matrix. This matrix (× 375) would be of about the same size as that shown in Fig. 77, A (× 250); and the spherical aggregates forming therein would seem to be similar to the spherules with which the body of the smaller Kolpoda was crammed that is shown in Fig. 77, C.

In Pl. viii., Fig. 76, we have illustrations of the Kolpoda matrices in their later stages of development. A (× 375) shows a matrix stained with carbo-fuchsine, which brings out the large nucleus that it contains. Its other contents are still merely granular, and show no arrangement into spherules—agreeing in this respect with the matrix shown in B (× 375), which can be seen to be segmenting into two, with the division passing through its nucleus. C (× 375) shows a large matrix dividing into four, with many of the characteristic spherules within, similar to what appear to be forming in Fig. 75, C. Both B and C were motionless, but D (× 250) is a poor representation of another cyst in which division had taken place into eight active embryos, and within the same cyst there were four or six Monads—an associa-

tion similar to what I have previously encountered on a few occasions (see p. 22). I could not determine the exact number of the Monads because of the way in which they were being tossed about by the eight active Ciliates, rolling within the thin, stretched, and almost invisible cyst. I ran some of a weak solution of mastzellen stain under the cover glass as quickly as possible, as I had found this to be one of the safest means of stopping the movements of these Ciliates without causing them to contract much. When they appeared to have come to rest I took the photograph, but its lack of definition shows that slow movements were still taking place. The Monads had in all probability been derived from a portion of the original matrix, which had in some way become separated therefrom. Such separated portions I have several times seen lying by the side of a matrix, and two of them are represented in Fig. 76, G (× 375), as parts of small matrices taken from an infusion of hay, in which they were found with a few other examples. E and F (× 375) shows two free Kolpodæ of unequal size. In the posterior part of the smaller specimen a dark nucleus is dimly seen, while partly overlying it, at the extreme posterior extremity, there is also a dim outline of the contractile vesicle. This specimen was killed with osmic acid, while the other unstained specimen in which neither nucleus nor vesicle is to be seen was brought to rest by a dilute solution of iodine.

In some of the matrices, as well as in the free-swimming organisms that issue therefrom, the body is more or less densely packed with small spherules. These are seen rather sparsely distributed through the substance of the matrix in Fig. 76, C, while in Fig. 77, A (× 250) they are very distinct within a very large matrix in which fission is commencing; and they are even more plentiful in the small free Kolpoda represented in B (× 375) which was stained by a dilute mastzellen solution and is shown in a state of contraction. In C (× 500) a part of the surface of an unstained, free-swimming Kolpoda is shown, whose body was densely packed with what are seen to be rather large, granular spherules; while D (× 500) shows a number of these spherules liberated from a very large Kolpoda by the dissolution of the organism (such as often takes place, as here, after the application of some stain), and at the same time the fact is revealed that the spherules when first formed are merely aggregates of Bacteria enclosed or bound together in some way.

It may be thought that these spherules represent the so-called stomachs of the Infusoria, and there is undoubtedly a certain resemblance thereto. But against this supposition there is the fact that they are often far more densely crowded together throughout the whole organism than these so-called 'stomachs' are ever found to be. Then there are the important facts that

they are as numerous in the matrices as in the free Ciliates, and that in these latter they are most abundant at the time when they emerge from the matrices. After living in the infusions for a day or two, with nothing but Bacteria to feed upon, these spherules instead of increasing in number gradually disappear. The spherules seem indeed to be analogous to the large corpuscles with which the bodies of Otostomas are crowded at the time when they emerge from the Hydatina egg-cases. The large corpuscles in these organisms also vary much in number in different specimens of Otostoma at the time of their emergence, and they always tend to disappear soon, and are mostly gone at the expiration of two or three days from the date of their birth. They seem to be digested in the case of the Otostomas; and the similarly speedy disappearance of the large spherules from the Kolpodæ after two or three days of active life may perhaps be accounted for in the same way. They would, in that case, subserve the same function as the stomachs, so that the only question that arises would be whether the Bacterial contents of the spherules were some of the original constituents of the matrices aggregated, as they appear to be aggregating in Fig. 75, C, or whether they have really been taken into the body during some previous free life of the Ciliates which present them. In addition to the other facts already cited against this latter notion there is the analogy of the crowded presence in the matrices, and the speedy disappearance after free life, of the large corpuscles in the Otostomas, and the certainty that these corpuscles have been fashioned in the matrices themselves—that is, from the substance of the Hydatina eggs (see p. 128).

The only Ciliates I have ever seen in the numerous hay or grass infusions made in the manner already described have been Kolpodæ, either large or small, in association with matrices similarly varying in size. These matrices, as I have said, appear in filtered infusions before the Ciliates. They appear in large numbers, in the course of a few days, in the substance of the pellicle. But in a few cases in which, in some early experiments, I added a small quantity of unboiled tap-water after the infusion had been filtered, another kind of Ciliate appeared in the infusions and rapidly multiplied therein.[1] In each case the Ciliates that appeared under these circumstances were of the same kind. They were Chilodons, such as are represented in Fig. 78, A (× 200) from a grass infusion. An enlarged figure of another of these Ciliates from a diluted hay infusion is shown in B (× 500), having its large nucleus stained, and the inner extremity of

[1] Of course if water is added for purposes other than mere experiment to test the effect of adding this or that kind of water (as referred to on page 100), the addition should always be of water that has been recently boiled.

its pharynx curved and hook-like. A similar condition of the pharynx existed in the others, and I have found it to be very common, though Saville Kent refers to such a disposition as a rarity.[1] I have never seen the least evidence pointing to the origin of these Chilodons in the grass or hay pellicles. No matrices of such Ciliates have ever been found in the pellicles when they have been present; nor have any of the Chilodons been seen in these infusions in a state of encystment. I have not the least doubt that the infusions were inoculated with the Chilodons when they were diluted with the unboiled tap water.

It is the more notable, therefore, that the other hay infusions with which I have worked, though prepared with the same kind of tap water and at temperatures under 100° F., should never have shown one of these Chilodons. It seems difficult to explain such a fact except on the supposition that the process of filtration had been for these infusions adequate to exclude all such Ciliates and any possible germs to which they may have given rise. But if the filter could thus exclude contamination by Chilodons, it ought to be similarly potent in excluding Kolpodæ or any bodies (nature unknown) which may act as their germs.

I have made very few experiments with other vegetal infusions, and shall only now refer to two of them, namely, to one prepared from Dutch Clover (*Trifolium repens*), and to one from Yellow Bed Straw (*Galium verum*), in both of which Ciliates were found in large numbers. Strangely enough, however, an infusion made from the Common Red Clover (*Trifolium pratense*), which had been growing side by side with the Dutch Clover, yielded no Ciliates, though it was prepared in the same way, exposed to similar conditions, and was examined as late as the twentieth day.

In the infusion made of the usual strength from the flowers, leaves and stems of the Dutch Clover a number of small matrices were found in the pellicle on the fifth day, having all the appearance of being aggregations of Bacteria enclosed by a delicate limiting membrane. This membrane soon became converted into a fairly thick cyst (Fig. 79, A, × 375). The matrices were situated in the deeper layers of a rather thick pellicle, and soon appeared in enormous numbers therein. Many of them closely massed together are represented in C (× 375); while one of the small Kolpodæ produced from one of these matrices is shown in B (× 375). Such Ciliates were soon found in the infusion in enormous numbers.[2] To later changes in this pellicle of an important kind I shall subsequently have to return.

[1] *Loc. cit.*, p. 747, Pl. xlii., fig. 20.

[2] Two infusions from other portions of this Dutch Clover, which had been kept in a card-board box for eighteen months, yielded similar Ciliates in like abundance.

The Yellow Bed Straw infusion was also prepared from the flowers, leaves and stems of the plant. Up to the seventh day neither matrices nor Ciliates were found in the pellicle that formed on the infusion; but when it was again examined on the eleventh day it was found to contain swarms of very minute matrices, and also multitudes of very small Kolpodæ. The subjacent fluid was almost clear but of a dark brown colour. The encysted matrices in their mature state were the smallest I have ever seen, and measured only about $\frac{1}{2000}$ inch in diameter. In Fig. 79, D (× 375) one is to be seen with only a thin investing membrane, but in the others this membrane has become thickened into a distinct cyst wall. By this eleventh day also there were multitudes of the smallest Kolpodæ I have ever seen. They had emerged from the diminutive cysts, and one of them is shown in E (× 375). They contained fine granules in their interior, and many of them had a rather truncated anterior extremity. In consequence of their minute size I measured them, and they were found to be about $\frac{1}{1150}$ inch long by $\frac{1}{2000}$ inch broad. Many of the matrices were seen dividing into two, but only one was found in which segmentation into four had taken place. Multiplication by fission seemed to occur almost solely in the embryos within the cysts (as with the Kolpodæ found in hay infusions), and only comparatively rarely, so far as I have seen, when the Ciliates were in their free state. The same kind of thing obtains with the Otostomas produced from Hydatina eggs.

The mode of origin of the matrices hitherto described, in which they are formed from aggregates of Bacteria or from individualised portions of the pellicle, of the actual size of the matrices ultimately produced after their enclosure within limiting membranes, is that by which such matrices first appear in properly filtered vegetal infusions. These are the primary matrices, which appear at periods varying mostly between three and eight days, and which, as I have said, remain stationary in size. This may be termed *the direct origin of Ciliates from the pellicle*, in contrast to another mode now to be referred to, which can perhaps be best spoken of as *the amœboid origin of Ciliates in the pellicle.*

In this second mode of origin the matrices, from minute beginnings, progressively increase in size, and when growth ceases generally become enclosed in thick and often brownish coloured cysts, which are rapidly formed and contrast notably with the often scarcely visible, pellucid cysts of the primary matrices.

This amœboid origin of the matrices reveals itself also at comparatively late periods, mostly from the tenth on to the twenty-fifth day; partly in the original pellicle, and partly in the

numerous zoogloeal, villous-like extensions therefrom into the fluid below.

They begin as minute corpuscles, which I have traced down to about $\frac{1}{5000}$ inch in diameter, and gradually increase in size up to that of small or medium sized matrices; though they never, I think, quite equal the very large primary matrices that may often be found taking origin directly from the substance of the pellicle. I call this "an amœboid origin" in the pellicle because these corpuscles, when they become larger than the tiniest specks, are seen to resemble embryo amœbæ in a resting stage. Their substance is pellucid with a few small granules scattered through it; and there is no appearance of their being aggregates of Bacteria such as we have invariably found to be the case with the primary matrices. No nucleus is to be seen in their interior; and they are spherical and unchanging in outline, though vacuoles occasionally appear in their substance. Growth continues up to a certain point, and when it ceases, the previously diaphanous limiting membrane becomes converted into a more or less thick-walled cyst.

In their early stages these amœboid corpuscles, destined to grow into ciliate matrices, have a very close resemblance to the discrete corpuscles which I have previously spoken of as developing either into Monads or Amœbæ. They are to be distinguished, however, by the fact that (*a*) the embryo matrices almost invariably stain of an old-gold colour with Ehrlich's eosinophile fluid, in the proportion of three or four drops to the drachm of distilled water, while the ordinary discrete corpuscles remain unstained; and (*b*) by the further fact that they are found not only smaller but also much larger than these latter corpuscles, and in aggregates the units of which vary much in size, instead of being practically all of one size, as the discrete corpuscles are found to be.

These various peculiarities in regard to the amœboid origin of the matrices are illustrated in Fig. 80, in which A (× 500) shows many of the coming matrices in their very earliest stages together with a few of them larger and more developed, taken from a hay infusion, on the eighteenth day, in which they were appearing and growing in the pellicle in myriads. B (× 375) shows three, each of them about $\frac{1}{3000}$ inch in diameter, unstained, from a hay infusion on the twenty-sixth day, and another of about the same size but stained with eosinophile. C and D (× 200) show a number of these amœboid matrices of larger size in the pellicle, and also some of the cysts of the kind to which they ultimately give rise, taken from another hay infusion on the thirteenth day. E (× 375), taken from a hay infusion on the eleventh day, shows two of the amœboid matrices just before, and two after, encystment, one of the latter containing a revolving embryo Ciliate (as

indicated in the photograph by its homogeneous rather than granular appearance). In F (× 250) an amœboid matrix just about to encyst is also shown to the right of the thick-walled, knobby cyst, such as it will speedily form. Multitudes of others were seen in intermediate conditions.

These secondary matrices, when mature, almost always develop comparatively thick cysts, and the embryos within them sometimes remain, for many days at least, in a motionless condition. As a rule, also, so far as I have seen, these matrices only rarely undergo segmentation; so that in regard to their mode of origin, their gradual growth, their formation of thick cysts, their quiescence for comparatively long periods, as well as this rarity of segmentation, the secondary matrices present characters strikingly different from those of the matrices that are developed primarily and directly from the pellicle itself or from aggregates of Bacteria forming therein.

I have principally seen two different kinds of cysts in these matrices of amœboid origin, one of them being thick and of brown colour, the surface of which is covered by brown, rounded knobbles. These have often been found in or on the under surface of old hay pellicles, somewhere between the fourteenth and the twenty-eighth days, and they vary much in size, as may be seen from Fig. 81, A (× 375), in which there is also represented an embryo contracted under the influence of formalin, which had just escaped from another of these cysts. The second form of late cyst, and the kind which is much the more common of the two, has not the same dark brown tint, but it has a wrinkled or plicated appearance, such as may be seen under a lower power in B (× 200). The formation of a cyst of this kind is common even in the segments into which the primary matrices divide, if the condition of the infusion becomes unfavourable to their existence in a free state. I have recently seen a large number of such matrices each of whose contents divided into two active embryos; but conditions becoming in some way unfavourable they speedily came to rest, and each segment assumed a spherical form within the original cyst. Two days later I found large numbers of these segments in plicated cysts rather than bounded by thin, smooth membranes as they had been previously (C, × 250); and often of two contained within the same cyst one had become so changed, while the other was still enveloped merely by the thin and smooth membrane which forms when the spherical shape is first assumed.

So far I have been speaking of the origin of matrices from amœboid corpuscles in the pellicle itself, where they commonly occur intermixed with others derived directly from its substance. I now have to speak of their appearance in the villi which, in an

old infusion, are apt to grow downwards from the pellicle.[1] Here, in these situations, away from atmospheric influence, matrices are formed only from amœboid corpuscles. I had often seen them swarming in such villi, and at other times I had found the villi containing an abundance of discrete corpuscles. Some of these are undoubtedly such corpuscles as are represented in Fig. 55, B, D, and which develop into Monads or Amœbæ, but I soon came to the conclusion that other of the corpuscles that appear in the villi are really embryo matrices of Kolpodæ.

I first obtained tolerably satisfactory evidence of this, and also as to the rate of change, during my examinations of the before mentioned infusion of Dutch Clover. On the nineteenth day I found that the villi which had grown downwards from the pellicle on this infusion contained a varying number of distinct spherical amœboid corpuscles, and one of these villi is represented in Fig. 82, A (× 250). Three days later some of the corpuscles found in other villi were found much larger, and more like embryo matrices. Six of such bodies, more or less in focus, are to be seen in B (× 250). Two days later still other villi were found containing a number of small but fully developed matrices, in about one half of which the embryos were revolving within their cysts. This was the condition of things in the portion of the villus represented in C (× 250), and also in another entire villus seen under a lower magnification, and stained, in E (× 60). Two of the mature matrices are also shown under a higher power in D (× 375) from the terminal portion of a villus.

Although different villi have been here represented, I have reason to believe, from the examinations I made of the state of many others of them at the intervals above indicated, that the examples I have given as to the rate of change, at three and two days respectively, fairly accord with the average rate of their development.

The presence of these later matrices in the villi, often in astounding numbers, was discovered almost accidentally. Only a very small portion of a pellicle remained on an old infusion prepared twenty-four days previously, which had been kept in a dark cupboard the whole time, and had not been looked at for the last eleven days. Before throwing it away I cut off a small portion of this pellicle, and to my amazement on examination found its villi crowded throughout with new Ciliate matrices, such as may be seen in the specimen shown in Fig. 83, A (× 15). The matrices varied much in size, and in the thickness (and consequently in depth of colour) of their brown cysts, as

[1] That is, where the pellicle is thick and formed on an infusion four or five inches in depth. A thin pellicle will not develop villous growths.

may be seen in B (× 60). Most of them contained embryos very slowly revolving; none of the cysts were empty, and no free Ciliates of any kind could be found in the infusion. The new brood had evidently not yet begun to emerge. C (× 60) shows how densely these cysts are massed together in some of the villi. No stain has been used for either of these specimens; the natural brown colour of the cysts causing them to appear as though they had been stained.

(2) On the Appearance of Ciliated Infusoria in Mixtures of Egg and Water.

I have made no observations worth mentioning on the pellicles forming on macerations of beef or mutton, or of the flesh of fish, because of the offensive putrefactions that occur in such mixtures.

The only animal mixtures on the pellicles of which I have made any lengthened study have been those formed by egg and water.

On several occasions I at first got very unsatisfactory results, and was deterred from making any but very brief examinations owing to the mixtures developing Bacteria in such extraordinary amounts, and to their rapidly becoming putrid. After a time, however, by making use of mixtures in a more diluted state, they remained comparatively inoffensive for several days, and I was thus enabled to do more work with them.

The strength finally adopted was one tea-spoonful of mixed yolk and white, to eight to ten ounces of water. I have used mostly eggs of the common fowl, but on a few occasions have also made use of Plovers' eggs. For the sake of comparison the mixtures have been made sometimes with ordinary tap water, and at others with water which has been passed through a Pasteur filter, or with distilled water. The water has been merely poured gently over the egg, or occasionally the egg has been stirred up with the water. Some of these mixtures have been kept indoors at temperatures ranging between 60° and 70° F., while others have been placed outside a window in summer (though not exposed much to the sun), covered with a cap of very fine wire gauze, so as to protect the mixture in part from dust.

The pellicle that forms on such a mixture is not a satisfactory one to examine. It is, in the early days, discontinuous and more or less mixed with vitelline granules in small masses; while later it becomes a very thick, tenacious and glistening mass of zooglœa, and the mixture also becomes distinctly offensive in odour.

On one occasion I used some of the 'white' of a fowl's egg alone, mixed with distilled water in the proportion of 1 : 3, and Monads were found to be present in great abundance on the fourth day. They have also generally appeared in mixtures made

with white and yolk at about the same time or rather earlier—and that when either kind of egg was used. But sometimes Monads have been absent when Amœbæ or Ciliates have been present in myriads. Thus, on one occasion when a mixture of fowl's egg and water was kept in the dark, in three or four days the pellicle was found to swarm with Amœbæ, as it continued to do when examined at times during another week, though neither Monads nor Ciliates were ever seen. At other times Monads would appear at first, and then, after three or four days, seem to give place to myriads of Amœbæ. The latter are seen best when portions of the pellicle are stained with logwood, and then somewhat compressed by the cover glass. Sometimes they are found scattered, and at other times dense aggregates of very minute, almost motionless, Amœbæ have been abundant, as though they were being formed from the segmentation of areas (as in Fig. 57, C, D). Up to the present, however, I have not been able definitely to satisfy myself in regard to the reality of this mode of origin in these mixtures of egg and water. At other times they seem to take origin in the pellicle as discrete corpuscles (Fig. 57, B).

In the observations I have made with Plovers' eggs, Ciliates appeared in large numbers on two occasions, while Monads were wholly absent. In each case, too, all the multitudes of Ciliates that were seen were Chilodons.

On the other hand, in mixtures of common fowl's egg and distilled water the Ciliates which have appeared on many occasions in great numbers have always been specimens of *Glaucoma scintillans*—a species which I have never met with elsewhere, during the last four years, except on one occasion when they were found in a mixture of Plover's egg and water. But even then the form of the Ciliate was rather different. It was more elongated than that which has so frequently been found in the mixtures of fowl's egg and water.

The Ciliates in these mixtures of egg and water have appeared when distilled water has been used, and when water passed through a Pasteur filter has been used; and on occasions in which Ciliates have shown themselves when ordinary tap water has been employed the form has still been *Glaucoma scintillans*, except that on one single occasion small Kolpodæ were found.[1]

This almost complete limitation of particular Ciliates to particular media is therefore in accord with the very striking fact previously brought out, that in filtered hay infusions, and in

[1] Glaucomas are in my experience extremely rare Ciliates outside egg and water mixtures. Only once during the last four years have I met with such organisms in other mixtures, and that was in association with one particular gathering of Euglenæ; even then the specific form was quite different from that of *G. scintillans* (see Fig. 85).

infusions of some other plants, it is Kolpodæ only that show themselves.

My observations with egg and water have as yet only been of a preliminary character; but so far it seems to me that the appearance of Ciliates in such mixtures is favoured by a comparatively free access of air and light, with exposure to temperatures of 60° to 70° F. Definite evidence as to the mode in which the Ciliates arise in such mixtures I have not yet been able to obtain, though I have made out a few facts of some significance to which I will now allude.

The only bodies having at all the appearance of being commencing matrices, in mixtures of the common fowl's egg and water, have been comparatively rare. Two aggregates of Bacteria which remained unstained, while Bacteria around took up the dye (magenta?) freely, are seen in Fig. 84, A (× 375). They were found in a mixture of egg and tap water after fifty-six hours. Several other curious aggregates of Bacteria being enclosed in coarsely granular cyst-like envelopes, such as are seen in B (× 375), were found in another mixture of egg and tap water on the fifth day. Their nature seems to me to be quite uncertain. In a mixture of egg and distilled water there were found on the fourth day two very large bodies which were undoubtedly matrices having thin, hyaline envelopes. Each of them stained of a delicate rose colour with an eosinophile solution. Each contained a number of large spherules very like those found in some of the Kolpodæ, and contractions of the embryo mass were distinctly seen though there were no movements of rotation. They were larger than, but in appearance very like, C (× 375), which shows a large embryo Glaucoma found on the fourth day in a mixture of egg and filtered water, in which these organisms were swarming. The Cilia were very short and scarcely visible; several spherules were seen within the body and also a large vacuole. In D (× 375) another Glaucoma from the same mixture but more fully developed is shown, having similarly short Cilia, and displaying, above and to the right, the mouth in profile.

A more fully developed form of *Glaucoma scintillans* is seen in Fig. 85, A and B (× 375), taken from another mixture of egg and distilled water on the fourth day. The Cilia are here much better developed. This organism was killed with a dilute iodine solution, and the photograph from which B has been reproduced was taken. The organism was then stained with eosine, and ten minutes later the photograph from which A has been reproduced was taken. This brings out the large nucleus very plainly. The site of the so-called anal aperture is seen in each of them. This organism was of about the average size, and was found to be $\frac{1}{500}$ inch long by $\frac{1}{625}$ inch broad. In their living active state

Glaucomas are egg-shaped, but when killed with iodine, as in D (× 250), they become rather more rounded in form. In respect of form, therefore, and also in regard to the great development of Cilia, the Glaucoma which on one occasion I found in association with Euglenæ, and which is shown in C (× 375), differs from the typical *G. scintillans*. The mouth, or vibratile lip of this new species is to be seen in profile above and to the left.

My observations with mixtures of Plover's egg and water have as yet not been numerous. In one mixture made with distilled water I found Monads abundant on the fourth day, and on the seventh day several matrices, but no free Ciliates. One of these matrices is shown in Fig. 86, A (× 375), and may be seen to be very similar to those found in hay infusions. It looks like a mere aggregate of Bacteria surrounded by a delicate membrane. It was motionless and showed no trace of a nucleus. The pellicle on the following day was found to be swarming with Amœbæ, and though a few matrices were found again on the fourteenth and up to the eighteenth day no free and active Ciliates were discovered. By the twentieth day the odour of the fluid had become extremely offensive and the pellicle sank. Too large a quantity of egg was contained in this mixture.

In another mixture of Plover's egg and water not a single Monad was to be seen on the seventh day though there were swarms of Ciliates which proved to be *Chilodons*—most of them immature, roundish or slightly ovoid, containing no contractile vesicle, but a large nucleus. They showed the characteristic pharyngeal armature, but only sparse cilia, and these so short as to be seen with difficulty, and principally about the anterior portion of the body. One of these immature forms is shown in Fig. 86, B (× 375), which had all the appearance of having been newly born, that is, of having just emerged from a cyst. These forms stained of an old-gold colour with a solution of eosinophile fluid. Some of them, quite spherical in shape, were found in the pellicle, but none were seen revolving. Only one was observed within a slightly stretched, almost invisible, hyaline cyst, in a specimen that had been stained with eosinophile. It looked as if the stimulus of the stain had made an immature Ciliate struggle; and that the delicate cyst had become stretched before the embryo had ceased to move. On the following day multitudes of these Ciliates existed in a fully developed condition; that is of a more elongated form, and with the characteristic lip-like expansion anteriorly, but having a more developed pharyngeal armature and longer and more abundant cilia. They were in fact now typical Chilodons, two of which are shown in C (× 375). No other kind of Ciliate was found in this mixture, and it is significant that the immature forms found on the seventh day

were nearly all of such a size as is represented in B, and that when examined two days previously no trace of a Ciliate could be found.

On another occasion in a mixture of Plover's egg and tap water no Monads were found, though after the sixth day the fluid swarmed with Chilodons. Twelve months later (that is last summer) being otherwise occupied I let the time slip by without again making observations with Plover's egg till it was almost too late, the season being over. I succeeded, however, in procuring one egg, and a mixture was made with water which had been passed through a Pasteur filter. On the third day I found in the pellicle on this mixture Chilodons and Glaucomas, fairly abundant and about equally numerous. On the following day the Glaucomas existed in enormous numbers, but only a very few Chilodons were seen. The Glaucomas too were larger and of a more elongated shape than those which had previously been found in mixtures of the common fowl's egg and water, as may be seen by D (× 250).[1] Their bodies were filled also with numbers of small granular spheres, and several of them were seen undergoing fission. On the fifth day, my note-book says, "the Glaucomas were still more numerous, composing about two-thirds of the bulk of the pellicle, but only one Chilodon was seen." So that in this, the one and only mixture of Plover's egg and water in which Glaucomas were found, they seemed to be of a new kind; they were at first found in association with Chilodons, but appear soon to have led to the disappearance of this latter form.

I fully recognise that these observations on mixtures of egg and water are too incomplete to enable us at present to come to definite conclusions in regard to the actual mode of origin of the Ciliates which are to be found therein. Still, all that has been ascertained as yet seems to point strongly to the conclusion that they also are produced in and from the constituents of the pellicles, in the same kind of way that Ciliates are produced in and from the pellicle forming on certain filtered organic infusions.

Some General Considerations Concerning the Origin of Ciliates in Organic Infusions.

In reference to the origin of Ciliates in filtered organic infusions there are five sets of facts of cardinal importance which must never be lost sight of. They are as follows:—

(1) It has been found that the different infusions are associated each with its own particular kind of Ciliate—that is where such

[1] It will be observed that the magnification is here lower than was employed for the representation of the other Glaucomas shown in Fig. 85, D.

organisms appear, for it must clearly be understood that they do not show themselves in filtered organic infusions with anything like the regularity that characterises the appearance of Bacteria, or even of Monads.

(2) Then, although the infusions after their preparation had been passed through the finest filtering papers, the Ciliates which first appeared therein in swarms (somewhere between the fourth and the eighth days) were, when they first appeared as free-swimming organisms, already of nearly full size—that is, even the smallest of them (in the infusion of *Galium verum*) had seven or eight times the diameter of the largest particle capable of passing through such filtering papers; while the larger specimens (found in hay infusions) had at least twenty times the diameter of such particles. It is well known that Swedish papers will not exclude particles of barium sulphate, and these, as I have found, have an average diameter of $\frac{1}{15{,}000}$ inch.

(3) Ciliates are not known to be reproduced by any very minute particles having the nature of spores or germs. Lankester says:[1] "Of the formation of 'spores' in this group we are at present ignorant, in spite of all that has been written on this subject." Similarly Saville Kent writes:[2] "Among the higher orders of the Class Infusoria sporular reproduction is comparatively rare, being as yet almost unknown among the groups of the Tentaculifera, while in that of the Ciliata a few stray instances only can be cited." It is, as he says, among the lower Flagellate Infusoria "that spore-formation attains its most vigorous development." The "stray instances" spoken of above by Saville Kent as occurring among the Ciliata are, however, quite unworthy of being considered as instances of spore-formation, seeing that what he refers to as instances of such a process is the fission of certain encysted Kolpodas, Otostomas, and one or two other forms, into two, four, or eight segments—that is, into masses which are often much more, and rarely less, than $\frac{1}{1000}$ inch in diameter, and are, from the first, small Ciliates. These are the only bodies that can in any sense be regarded as "spores" among the Ciliata which have for years been the subject of numerous and most elaborate investigations by skilled observers. Their only known modes of increase hitherto have been by processes of fission, occurring either in their free state or when encysted, and, though much more rarely, by a process of gemmation.

(4) But it has been found that the Ciliates which first appear in our filtered organic infusions as nearly full-sized, free-swimming organisms are always preceded in the pellicle by encysted matrices from which they emerge. These matrices could never, as such,

[1] Introduction to translation of Gegenbauer's "Comparative Anatomy," 1878, p. ix.
[2] "Manual of the Infusoria," 1881, vol. i, p. 89.

have passed through the filter; and even if they had done so, looking to their size and weight, they would have accumulated at the bottom of the vessel rather than at the surface of the fluid. They could not have got into the pellicle at all unless they, or the bodies from which they had been derived, had previously enjoyed an active life. But, as I have said, the matrices appear in the infusions before the Ciliates.

(5) In the face of these facts we are driven, therefore, to the conclusion that the primary matrices must be formed from the pellicle itself. And that is what I have shown to be actually the case. The early stages of these matrices have been demonstrated to be mere aggregates of Bacteria similar to those existing in the pellicle lying around them, each of which becomes enclosed by a bounding membrane, develops a nucleus, and then becomes evolved into an active embryo Ciliate. This embryo may be seen revolving within its cyst, previous to rupturing it and moving away as a free-swimming organism.

This mode of origin of Ciliates from the pellicle is, moreover, in exact accordance with what I have abundantly shown to be the mode of origin of embryonal areas in and from the pellicle, and also with one of the modes of origin of Monads. We are alike incapable of explaining either set of facts, and however indisposed naturalists may be at first to believe that these interesting and much-worked-at forms of life—the Ciliata—can have such astounding and comparatively rapid modes of origin, yet their origin in this way is in reality no more astonishing and apparently incredible than the origin of embryonal areas from aggregates of Bacteria, the fusion of such aggregates into individualised masses of matter, followed by repeated processes of segmentation, ultimately resulting now in the production of Fungus-germs, now of Monads, and now of Amœbæ.

Even if this last and final proof as to the origin of the Ciliates in filtered organic infusions had not been made out, the other facts mentioned would have been, as I have said, almost impossible to be explained satisfactorily, except on the supposition that the Ciliates had been formed in some such manner from the pellicle itself.

For if the first Ciliates that appear in infusions (of which we are now speaking) came from the air or the water there ought not to be this constant association between the particular kind of Ciliate found and the material from which the infusion has been derived. And, again, if the forerunners of the Ciliates had passed through the filter as invisible germs derived from air, water, or the plants themselves, the Ciliates ought not to reveal themselves all at once (or comparatively so) as organisms of full size. All sizes between such hypothetical invisible or minute germs and the full-sized organisms ought to be forthcoming. They

are, however, absent; and, as we have seen from the previous quotations, there is no ground for supposing that any such minute germs or spores are ever formed by Ciliated Infusoria.

These difficulties taken together, as well as the others previously referred to, are, therefore, incapable of being reconciled with the facts, unless it could be shown that the Ciliates which first appear are developed in and from the substance of the pellicle itself. That being so, it would be comparatively easy to understand particular Ciliates always appearing in particular infusions, and appearing, almost at once, nearly of full size.

So much then concerning "the direct origin of Ciliates from the pellicle"; but, as I have already shown, that is not the only way in which they arrive in organic infusions. We have also to bear in mind what I have termed "the amœboid origin of Ciliates in the pellicle." I am not altogether satisfied with this latter designation, and yet am unable at present to find a better phrase. All that I mean to convey by such a designation is that the Ciliates in these cases take origin from very minute corpuscles which are at one stage of their existence almost indistinguishable from the discrete corpuscles that develop into Monads or Amœbæ; and that these corpuscles go on increasing in bulk, always having very much the appearance of Amœbæ in a resting stage, till they attain varying sizes ranging from about $\frac{1}{2000}$ inch to $\frac{1}{800}$ inch—when they cease growing, develop more or less thick cysts, and become themselves converted into embryo Ciliates, which after a time may be seen revolving within their temporary prisons.

These amœboid corpuscles stain of an old-gold colour with a dilute eösinophile fluid, while the ordinary discrete corpuscles which develop into Monads or Amœbæ exhibit no such reaction. Then again, these latter corpuscles are formed from the pellicle of about the same size as the Monads and Amœbæ to which they give rise; they do not grow in bulk, they only develop. But the corpuscles that ultimately develop into Ciliates begin as very minute particles, which I have been able to trace by the aid of stains down to $\frac{1}{5000}$ inch in diameter; and they grow enormously, till they attain such sizes as I have above indicated, previous to encystment.

The two modes of origin of Ciliates agree in this important respect that in each case the Ciliates are actually formed as such within cysts, and that in each case also the free-swimming organisms emerge from the cysts approximately of full size.[1]

As to the actual source or origin of these minute particles

[1] Such embryos were found in the egg and water mixtures (Figs. 84, C, and 86, B); and they will also be met with in connection with the Vorticellæ to be described in the next section (Fig. 88, D) as they were also shown in Pl. iv., fig. 42, E).

which appear after many days in the midst of the pellicle or of its outgrowths, and go on increasing in size till they become encysted masses, each of which gives origin to a Ciliate, nothing definite can at present be said. It may be surmised by some that they are germs or minute spores derived from some previous Ciliates. But against such a supposition various weighty considerations may be urged.

In the first place, as we have seen, no such germs or spores are known, so that the supposition would be opposed to all existing knowledge.

Then there is the fact that these bodies only show themselves after ten or fifteen days, and even then only when we have to do with infusions four or five inches in depth, or so strong as to be capable of forming thick pellicles from whose under-surface numerous villous outgrowths project. In such zooglœal outgrowths, or in the substance of the thick pellicle itself, these minute Amœboid bodies appear in myriads and go on to the production of Ciliate matrices such as we see in Figs. 80, 82 and 83; while in a weak or a shallow infusion, forming only a thin pellicle, no such bodies are prone to occur.

Then, again, in the cases where these minute amœboid bodies arise and go on to the formation of Ciliate matrices, is it conceivable that myriads of them, always of precisely the same kind, should occur in each particular infusion if they were due to external contamination? It cannot be supposed that their vast numbers are due to self-multiplication, for such particles are motionless, and through their various stages of growth can never be seen to divide till after they have attained their full size and have become converted into embryo Ciliates.

There is the further important fact, telling strongly against the origin either of the primary or of the secondary forms from external contamination, that Ciliates only appear in filtered infusions prepared from absolutely ripe or from dead grasses, and not from immature grasses in the fresh state, as I have shown on p. 87. If contamination came from earth, or air, there seems absolutely no reason why it should not come with fresh immature grasses, as well as from ripe, dead grasses.

V. ON THE EVOLUTION OF VORTICELLÆ IN AND FROM A PELLICLE LARGELY COMPOSED OF SPIRILLA.

Some Euglenæ, together with sewage-tainted water from a farm yard in which they were found, were in November, 1899, placed in a large shallow vessel, in which the water stood at a depth of about one inch. The surface of the fluid soon became covered with a thick coating of Euglenæ, some of which were

removed for examination over an area of about two square inches.

By the tenth day that portion of the surface of the fluid from which the Euglenæ had been removed was found to be covered with a fairly thick Bacterial pellicle, very largely composed of Spirilla, a portion of which is shown in Fig. 87, A (× 375). In this pellicle aggregations of the Spirilla and other Bacteria were found, during the next two or three days, of such a kind as to form more or less spherical heaps about $\frac{1}{800}$ inch to $\frac{1}{600}$ inch in diameter. Two of these aggregates, lightly stained with magenta, are shown in B and C (× 375). Another of these bodies is to be seen in D (× 375) as a more neatly-defined spherical mass of Bacteria, though with no distinct limiting membrane. In E (× 375) we have a more advanced stage in which the mass of Bacteria is now bounded by a limiting membrane, which has been rendered particularly distinct by a staining with logwood. The dye in this case has not penetrated to the Bacterial contents, though in a specimen by its side, unprovided with a limiting membrane, the whole mass of Bacteria had become stained, as in D.

In Fig. 88 the later stages in the development of this kind of matrix are shown. In A (× 375), which was lightly stained with magenta, the embryo has become more distinctly organised; while in C (× 375) we have a still more mature embryo, whose mass is now distinctly separated from its enclosing cyst. D (× 375) represents a specimen lightly stained with eosine, and shows a free embryo together with the cyst from which it has just emerged under the stimulus of the dye. B (× 375) shows another of these embryos (very deeply stained with magenta) just ready to emerge from its cyst, and by its side the outlines of one of the Vorticellæ which has been produced from such an embryo.

In Fig. 87, D, we have on the left side for purposes of comparison one of the developed Vorticellæ which, after increasing in size and living an active life for a time, has now become encysted. It was found, as may be seen, by the side of one of the matrices in process of formation, and had, like it, been immersed for a short time in a logwood solution. In the specimen itself a hyaline and almost invisible membrane (not shown in the photograph) was seen just outside that which is represented. The smaller matrices, the stages in whose development have been shown, are therefore totally different in size, as well as in other respects, from the spherical bodies produced by the encystment of the Vorticellæ after they have grown and for a time lived an active life.

These Vorticellæ soon became excessively numerous in this small pellicle. One group of them is represented in Fig. 89, A (× 80), after it had been in a weak eosine solution for an hour. Some of the Vorticellæ had become darkly stained, others lightly,

and others not at all, though even the latter had been rendered motionless, and were therefore capable of being photographed. The eosine only stains such organisms deeply (as well as Monads and Amœbæ) after their death. At first it decidedly stimulates them, as may be seen by their more rapid movements. B (× 200) shows other of these Vorticellæ, more enlarged, from the same slide.

The stages by which such spherical matrices as are shown in Fig. 88 became developed into Vorticellæ were seen and fully described in 1872,[1] though in that case the matrices had been derived from the transformation of Euglenæ. It has also been followed in part, and referred to on p. 46, when speaking of the Vorticella matrices that were produced within Hydatina eggs.

This new origin of Vorticellæ from mixed aggregates of Spirilla and other Bacteria (though it harmonises pretty closely with what we have seen occurring in the production of Kolpodæ, and in the production of 'embryonal areas' from the pellicles on hay infusions) is as totally unexpected and inexplicable as either of their other modes of origin above referred to.

VI. ON THE TRANSFORMATION OF ENCYSTED EUGLENÆ INTO CILIATED INFUSORIA.

Many different forms of Ciliates are at times produced by the transformation of encysted Euglenæ, kept under unnatural conditions. When, for instance, a gathering of Euglenæ is put into a vessel of water and left exposed to light and air outside a window, in twenty-four hours or less, should the weather be bright, we may find a beautiful green layer uniformly covering the surface of the fluid. If we examine a portion of this scum with the microscope we may see only Euglenæ side by side, forming a sort of tesselated carpet. If such a vessel is then brought indoors and placed under a large glass shade, the Euglenæ in the dimmer light soon become encysted, and in the course of five or six days certain of them begin to undergo various transformations, some of which I have already described (pp. 11-13 and 19).

Another kind of change which is very common is the gradual decolourisation of the Euglenæ—the green contents becoming bit by bit converted into colourless protoplasm, till at last the red eye-speck, together with some refuse reddish-brown pigment granules, are alone left unconverted. Shortly, however, these also disappear and we are left with a whitish colourless mass of protoplasm as the representative of the previously bright-green Euglena with its red eye-speck. Such changes are exceedingly

[1] "The Beginnings of Life," vol. ii., pp. 462-466.

common, so that after two or three weeks, when we examine another portion of the scum we may find instead of the uniform layer of green juxtaposed Euglenæ, that numbers of them have been converted into, and are replaced by, spheres of colourless protoplasm produced in the manner I have above described, and presenting such an appearance as is shown in Pl. ix., fig. 90, A (× 80).

These transformed Euglenæ remain for various periods without undergoing any very appreciable changes—it may be for days only, or from two to twelve weeks, according to the conditions to which they are subjected. As a rule such colourless matrices do not increase in size, but after undergoing certain molecular changes they may develop a contractile vesicle, and begin (at first almost imperceptibly) to revolve within their cysts; though later, as cilia become more developed, their movements of revolution increase in rapidity.

In more exceptional cases the colourless encysted matrices increase to a variable extent in bulk, and when this growth ceases the cyst may become thicker and develop spines on its exterior, such as I have represented in Pl. iii., fig. 25. From such cysts Stylonychiæ were often seen to emerge.

The retrograde changes in some of these Stylonychia matrices by which they were converted into flagellate Monads or Peranemata have been already described (pp. 26-29); but the question of their origin was deferred. All that was said on the subject of the origin of these spinous cysts was this: "I will only say that I have found many thousands of them in association with a gathering of Euglenæ; and though I have seen many specimens of Stylonychiæ come out of them I have never seen a single one of them formed by the encystment of such a Ciliate. They appear rather to have been derived from much smaller matrices, found in association with the more developed specimens, which gradually increased in size and formed a thicker envelope upon which the spinous processes were finally developed. The mature specimens varied a good deal in actual size."

Such bodies as are to be seen in Fig. 90, A (× 80) when they have become slightly increased in size and show indications of a nucleus, are represented, under a higher power, in B (× 250). In C (× 250) two of these bodies are shown which have still further increased in size, and the one on the right may be seen to have begun to develop spines from the outer surface of its cyst. Such spines fully developed are shown in D (× 250), in which the surface of one of these cysts has been focussed. E (× 250) represents another of these encysted bodies which had just begun slowly to revolve within its cyst. When I proceeded to photograph it no movements were appreciable,

but these must have been stimulated under the strong light from the lamp, the result being the homogeneous appearance shown in the photograph. The body that emerged from such a cyst, after a series of violent movements, is shown in Fig. 25, B (× 375), as a young Stylonychia.

In other cases the transformation into Ciliates of Euglenæ which come to rest on the surface of the fluid, and within cysts so thin as to be scarcely appreciable, has been seen taking place much more rapidly. I have traced all the stages in the formation of Nassulæ in this way, and will now describe and illustrate the mode in which the transformation has been effected.

Towards the end of November of last year, after two days of slight frost, I procured from a ditch some damp mud on which there was a coating of Euglenæ. A portion of this was placed in a small vase, which was then filled with water. The vase was left on the end of the mantelpiece in my study nearest the window, covered by a glass shade, which was allowed to overlap the edge of the mantelpiece, so as to permit access of more air to the Euglenæ than would otherwise have been the case.

When examined three days later a thin scum composed in the main of small Euglenæ was found on the surface of the fluid. A few of the Euglenæ were seen which were becoming decolourised, and exhibiting different stages of the process. They were all motionless, round or oval, and of just the same varying size as the unaltered Euglenæ among which they were situated. During my examination of the pellicle, many flagellate Monads and Rotifers were seen; but no Ciliates of any kind were met with. Two days later the decolourised Euglenæ were found to be still more numerous, while many of the unaltered Euglenæ were slowly rotating within their thin and scarcely visible cysts. Twenty-four hours later, that is, on the sixth day after gathering the Euglenæ, I found a few of the decolourised specimens showing traces of the formation of the characteristic buccal cylinder of Nassulæ, and a few days later I saw such Ciliates revolving within, and issuing from, their cysts.

These several stages of the transformation may be illustrated by the photographs comprised in Pl. ix., fig. 91. A (× 375) shows a group of five Euglenæ in different stages of decolourisation. In the two lower specimens the change is most advanced, and in the one on the left it was complete except for one small mass of the green substance. B (× 375) shows three other Euglenæ completely decolourised except for a few green granules remaining, together with a vestige of the red eye-speck in the upper specimen. That on the right shows a small vacuole; but in neither of them is any trace of the buccal tube or of a nucleus to be seen. In C (× 375) the first rudiments of the buccal tube

are to be seen appearing in two of these transformed Euglenæ, and in another specimen in which the tube seemed to be in about the same stage of development an ovoid nucleus was seen, though I did not succeed in photographing it satisfactorily. D (× 375) shows one of these Ciliates, which was killed by a very dilute formalin solution, just after its emergence from one of the cysts. It seems large in proportion to the size of the cyst from which it has escaped—as is generally the case both with Ciliates and with Rotifers at the time of their emergence from cysts. It bears, however, the usual marks of immature development. This is indicated by its delicate texture and by the scanty development of a few weak Cilia about its anterior extremity, as well as by the comparative absence of contents, permitting the nucleus and the now more developed buccal cylinder to be so plainly seen. In twenty-four hours or so the appearance of the organism becomes distinctly altered, as may be seen in the specimen shown in E (× 375), in which short Cilia had developed all round the body, and in which the larger amount of contents hid the nucleus and in part also the buccal cylinder. Later on, when such organisms become fully developed, they prove the most ravenous creatures, and may often be seen gorged with food of different kinds — sometimes Diatoms, sometimes Oscillatoriæ (which may be taken in so as greatly to distort their bodies), and at other times with green algoid corpuscles, such as may be seen in F under a lower degree of enlargement (× 250).

On other occasions I have seen Chilodons produced from Euglenæ by a very similar set of changes. Transformations of this kind, as well as into other forms of Ciliates, or into Amœbæ, are also prone to occur when portions of a fresh Euglenæ pellicle are transferred to the surface of water in a stone pot, on which the cover is subsequently placed. When portions of this pellicle are examined after an interval of seven, ten or more days we may find transformations of the kind indicated occurring in some of the Euglenæ, which have thus been left not only cut off from all the rays of light but in a very confined air space.

VII. ON THE SEGMENTATION OF SOME ENCYSTED AMŒBÆ, AND ON THE CONVERSION OF THE SEGMENTS INTO CILIATED INFUSORIA OR THEIR RESOLUTION INTO MONADS.

It has been known since the investigations of Spallanzani that tufts of moss and lichen are tenanted by three kinds of organisms, all of which have the power of reviving after even prolonged periods of what appears to be complete desiccation. The animals recognised by him were certain Rotifers, a few Nematoids, and some of the curious group of Tardigrades, to

which Spallanzani gave the name of 'Sloths.' When working at the 'Free Nematoids' in 1864 I repeatedly found these same organisms in moss and lichen from the most varied sites, and ascertained that the Nematoids met with, in most cases, belonged to one or other of two genera (Plectus and Aphelenchus) several species of which were described in my "Monograph on the Anguillulidæ or Free Nematoids, with Descriptions of 100 New Species." [1] In the following year I again gave some attention to this subject and showed in a memoir on "The Anatomy and Physiology of the Nematoids, Parasitic and Free," [2] that the ability of the Nematoids found in moss and lichen to recover after prolonged desiccation was by no means shared by the 'Free Nematoids' generally.

I was much struck then with the singular fact of the invariable presence of these three kinds of organisms—Rotifers, 'Sloths,' and Nematoids—in all the specimens of moss and lichen examined, quite irrespective of the sites in which they had been growing. And my surprise was all the greater because each of these forms of life is only known to multiply by comparatively large eggs, such as have scarcely ever been found in the atmosphere when its corpuscles have been collected and scrutinized.

I have long had the intention, therefore, of examining more thoroughly the different kinds of organisms that are to be found in these situations, taking especially the orange-coloured lichen, *Parmelia parietina*, as that was one I had frequently examined in 1865, and was also one which could be obtained free from minute sand particles, the presence of which would make the examinations of its fauna more difficult. When in the Pyrenees last autumn I found this lichen growing in great profusion on the bark of fine poplars forming an avenue in the Allée de la Pique, at Bagnières de Luchon, and I brought away many specimens thereof. Some months after my return I, from time to time, placed small portions of this lichen on a microscope slip to which a few drops of distilled water were added, and after allowing it to remain in a damp chamber for ten or twelve hours the fragments of lichen were removed, and a large cover-glass was placed over the fluid and what it contained.[3] Examination with the microscope often showed the presence of one or other, and often both, of two Rotifers belonging to the genus Callidina, of Nematoids belonging to the genera Aphelenchus and Plectus as well as others, and also a few 'Sloths' of the genus Macrobiotus. In addition to these organisms, however, there were also a number of Ciliated Infusoria (the most abundant after this

[1] *Trans. of the Linnean Society*, 1864, vol. xxv., p. 86.
[2] *Philosoph. Trans.*, 1866, pp. 613-619.
[3] It is at this stage that sand particles, when present, become troublesome by hindering the proper application of the cover-glass.

short period of soaking being Kolpodæ), a quantity of small Flagellate Monads, and occasionally certain large, sluggish Amœbæ.

Subsequently I allowed fragments of the lichen to lie with their under surface in distilled water in a shallow glass vessel (protected by an inverted claret glass) for six or even ten days, and then examined, drop by drop, portions of the fluid and of the sediment which it contained, gathered by means of a tiny pipette. After this more prolonged soaking several other kinds of Ciliated Infusoria were often found; and more of the Rotifers, Nematoids, 'Sloths' and great Amœbæ were obtained.

This seemed the only way of attaining a knowledge of the kinds of organisms existing in and on the under surface of the lichen, but it is a tedious process and one requiring much labour to examine every portion of the contents and sediment in half a drachm or more of fluid left after each soaking. Even then, of course, one only encounters the organisms which have dropped away from the lichen, while very many other would almost certainly remain attached to its under surface or concealed within its interstices.

It will doubtless seem to many a strange place in which to find different kinds of Ciliated Infusoria fairly abundant, seeing that this lichen grows most plentifully on the bark of the trees in situations where it is exposed to the full glare of the sun. Some of the same trees which chanced to be growing in the shade had their bark pretty thickly covered with moss rather than with lichen, and the few examinations that I have made, in the manner above described, of portions of this moss have shown that its fauna is distinctly less varied and less abundant than that existing in the lichen.

The large Amœbæ, with which we are now concerned, found in the sediment left after the soaking of the lichen, are always extremely sluggish, and are often crammed with food fragments and particles composed of portions of the under surface of the lichen, having a brown, red-brown or orange colour, the substance itself of the Amœbæ being at an early stage of the digestive process often more or less colourless. Such a specimen is shown in Pl. ix., fig. 93, A (× 150). After a time, however, much of this food-stuff becomes digested, and then the Amœba presents a pretty evenly granular structure, but is often of a pale red or even of a blood-red colour except at its mere margin or where it throws out coarsely-lobate projections as in Fig. 92, A (× 150). These projections are generally perfectly hyaline and translucent, contrasting notably with the blood-red colour of the great bulk of the organism. Sometimes large quantities of undigested refuse, in the form of spherical pellets of different sizes, are

extruded, and the Amœba may begin to encyst itself at once, as in the pale red specimen represented in Fig. 92, B (× 200). The movements of these creatures are so extremely sluggish that I was able to take the foregoing photographs while they were still living.

These Amœbæ are not always of a pale red or dark red colour; some are nearly colourless, as in the small specimen shown in Fig. 94, A (× 200). Such variations depend upon the portions of the lichen upon which they have been feeding—some of the under surface being whitish, while other parts are of a red-brown colour. The undigested refuse pellets may be all extruded before, or not till after, the Amœbæ have begun to encyst themselves. The cyst itself is extremely thin and apparently somewhat glutinous, as particles of different kinds and some of the small refuse pellets are generally found adhering to its outer surface, as in Fig. 94, A, and Fig. 92, B. In the latter specimen digestion had only been partially accomplished, as there were food-masses still within as well as outside the pale red body-substance; while in the former smaller, and unusually pale, organism digestion was almost completed.

Another of these pale red encysted Amœbæ was found which had divided into eight or ten unequal spherical segments, such as may be seen in Fig. 92, C (× 200). Only five segments are distinctly to be made out in the photograph, but under examination by the microscope others were seen at lower levels. They were all motionless, granular masses of protoplasm—probably about to be evolved into Ciliates, densely packed with large pale red corpuscles, such as have been seen actively swarming about within other of these Amœba cysts. One of them containing six dark red and very active Ciliates is shown in Fig. 92, D (× 200), after the movements of the Ciliates had been brought to rest by means of a weak osmic acid solution. Both of these cysts had an abundance of the usual minute particles adhering to their outer surface. Another rather larger cyst, containing six very active, blood-red Ciliates, is shown in Fig. 92, E (× 200). Other similar specimens have also been found in which there have been the same kind of Ciliates, more or less red, within these flexible cysts, coated externally with a large quantity of adherent foreign particles.

These red Ciliates, in their immature condition, as found within the cysts, are slightly pointed at the anterior extremity, where short cilia are principally developed. Their bodies have been so closely packed with red corpuscles that I have hitherto quite failed to make out the shape and situation of their mouth, and I have been unable to preserve them long enough in their free state, beneath the cover-glass, to allow these corpuscles to disappear. They doubtless would disappear in the course of a

day or two, just as the very similar corpuscles found in the bodies of the immature Glaucomas described in the next section disappear during the time needful for the attainment of their adult form.

We have here another very important mode of origin of Ciliates of a kind not previously suspected. The orange-red colour, more or less marked, of the Amœba in its free and in its encysted state; the character of its cyst; the finding within such a cyst a number of motionless but unequal red spheres, into which the encysted mass had separated; and within others the existence of a number of similarly unequal, active, red Ciliates—constitute a set of facts which can only be interpreted by the supposition that the red Ciliates have been developed from the red Amœbæ after they had become encysted and had undergone segmentation.

But another still more convincing and remarkable illustration of this new mode of origin of Ciliates has also been encountered in some of the largest specimens of these encysted Amœbæ. The cyst has been of the same kind and similarly coated with a quantity of minute adhering particles. One of them is shown in Fig. 93, B (× 200) entire and of a dark brown colour, which, under examination with the microscope, was found to be densely packed with a multitude of small, motionless spherical bodies. When found, I knew at once the nature of the specimen, because I had previously discovered two others, and one of them I had seen burst open under the weight of the cover glass and give exit to multitudes of small spheres of a light, red-brown colour. D (× 200) represents one of these cysts after very many of the contained spheres had flowed away; while C (× 250) shows a portion of the other at a slightly higher magnification after nearly all the spheres had been squeezed out of it. This illustration is intended to show the nature of the brown cyst in which they were contained, together with its adherent particles.

A careful examination of these spheres made it obvious at once that they were small Ciliate matrices almost exactly like, in colour as well as in size, those found in the infusion of Yellow Bed Straw. In E (× 375) these matrices are represented under the same degree of magnification as those shown in Fig. 79, D, which were proved (p. 96) to be matrices of small Kolpodæ. When first seen a very few of these matrices from the encysted Amœbæ were found to contain what was either a pale nucleus or a contractile vesicle, and they were all motionless—and so they remained during several days in which they were kept beneath the cover-glass in a damp chamber. I then transferred the remains of the two cysts to some water in a covered watch glass,

though the unopened one was unfortunately lost during my attempt to place it under similar conditions more favourable for the development of its contents. This cyst was obviously like the other two, only the contained matrices were distinctly larger, and therefore not so numerous—while the cyst itself was smaller.

After the cysts had remained in the watch-glass for a week, I took them up with a tiny pipette for examination. The matrices, however, were still motionless, and no small Ciliates were seen. They were then allowed to remain beneath the cover-glass, and kept thus in a damp chamber for another week, and by this time an increasingly large number of them showed what I believe to be a vacuole, rather than a nucleus—as in those seen in E (× 375). The nucleus in such Ciliate matrices is not often visible without the aid of reagents, but a vacuole is often to be seen which seems to be the precursor of the contractile vesicle. It is often seen, for instance, in the matrices of Vorticellæ, showing no contractions; and when, after a time, contractions begin they occur only at comparatively long intervals of two to three minutes. Many Ciliate matrices remain for weeks without undergoing any change; and these specimens within the two cysts seemed not likely to make any further advance under the unfavourable conditions in which they were placed. The substance of many of them was, in fact, becoming diffluent, so they were no longer kept under observation.

One of the most notable things in regard to these specimens was the very great number of the Ciliate matrices which were derived from a single Amœbæ. There certainly must have been over one hundred in each of the two cysts that were ruptured. Another notable fact is the small size, and the comparative equality in the size, of the matrices—points in regard to which they differ from those shown in Fig. 92, and from other of these cysts where I have seen that the encysted Amœbæ had separated into a much smaller number of larger and very unequal segments. This same inequality of size, though on a smaller scale, has also been seen during the transformation of some Rotifers' eggs into Amœbæ and Peranemata (pp. 31 and 37), and in each of these cases the parent mass appears to separate simultaneously into the various unequal segments—they are not produced by a succession of binary divisions, like that which occurs during the normal development of the egg of a Nematoid.

Some of the smaller encysted Amœbæ, such as are shown in Fig. 94, when kept under adverse conditions—that is, beneath a cover glass in a damp chamber—have undergone transformations of a different order. For instance, one of these small encysted Amœbæ was found divided into four unequal segments, each of which was comparatively colourless and finely granular,

as shown in Fig. 94, B (× 200). It was left beneath the cover glass and placed in a damp chamber on the mantelpiece. Twenty-four hours later the segments had become of a slightly brownish tint (such as I have seen Ciliate matrices assume during development) and there was also the appearance as of globules forming within the segments (C, × 200). The specimen was replaced in the damp chamber and, when examined again three days later, two of the segments were found to have divided into a number of small, pale, refractive, spherical bodies, as in D (× 200). After another twenty-eight hours in the damp chamber, the little spherical bodies had disappeared from the two segments—apparently as small flagellate Monads such as were now to be seen in their neighbourhood—while the remaining two segments had become resolved in a similar manner into motionless Embryo Monads, as shown in E (× 200).

The appearance of the Monads, as well as their slow mode of formation, sufficed to completely differentiate them from the Zoospores of some Fungus. Moulds have, moreover, never been seen in any of my examinations of the products obtained from the lichen. I am inclined to think that the resolution of the segments of the Amœbæ into Monads may have been owing, in the main, to the conditions to which they had been subjected. A small entire cyst thickly covered with particles of different kinds is shown in F (× 200), which contained not Monads but five or six active red Ciliates—though since the application of osmic acid the outline of only one of them in the upper part of the cyst is now to be distinctly seen. The mere small size of the pale encysted Amœba was, therefore, probably not an influential factor in determining the resolution of its segments into Monads rather than their development into Ciliated Infusoria. Still it cannot be said that the keeping of the specimen for some days beneath a cover glass was with any certainty the cause of the resolution of the segments into Monads; for although the other specimen, in which Ciliates developed, had not been kept beneath a cover-glass there was another point of difference—the parent mass had been derived from a red rather than from a colourless Amœba, so that some difference in molecular constitution may have been, at least in part, the cause of the difference in the products in the two cases.

VIII. ON THE TRANSFORMATION OF THE SUBSTANCE OF THE EGGS OF ROTIFERS OF THE GENUS CALLIDINA INTO A NEW KIND OF CILIATE BELONGING TO THE GENUS GLAUCOMA

As I have already intimated, in my examination of the organisms found in the distilled water in which the *Parmelia parietina* from Bagnières de Luchon had been macerated, I have frequently found two species of Rotifer belonging to the genus

Callidina.[1] The more common form has been *C. constricta*, while the other, *C. vorax*, is larger, and has a body marked by several longitudinal ridges. Both living and dead specimens of each variety have been commonly met with; and in the latter, as well as in the former, eggs have frequently been found. In the living specimens, for the most part, only one mature egg is to be seen; while in the dead specimens, two, three, or even four may be found.

In Fig. 95, all the components of which are magnified 250 diameters, different specimens of these dead Callidinas are shown. A represents a specimen with a single mature egg which has not yet begun to develop; while B shows another whose body is almost completely filled by four equal-sized eggs all of which present the appearance commonly met with in an early stage of normal development. And that the normal development does go on at times within the dead body of the parent is evidenced by C, which represents a young Rotifer, now contracted under the influence of osmic acid, which previously had been seen in active movement within the body of its parent, prodding first at one extremity and then at the other in its efforts to escape from its prison. D shows another of these Rotifers containing two eggs, one of which is simply mature, while the other is much changed, and in a manner that I have never seen when the normal process of development has been proceeding. E, again, shows another Callidina much disintegrated but containing two eggs which I also think are undergoing some abnormal changes.

In other cases only Ciliates, and no eggs, have been found within the dead Callidinas. Thus Fig. 96, A (× 250) shows a single large active Ciliate within the dead Rotifer; almost all that is left of the Rotifer being the jaws and integument—the former loose and driven about by the active Ciliate. B (× 250) shows another large specimen, nearly half filling the body of the smaller Rotifer, which, while alive, was plainly seen to be in an early stage of fission—though the position now represented does not show it. In C (× 250) a specimen is seen in which fission into two has taken place, though when it was first observed six hours previously, this Rotifer contained only a single large Ciliate. In the interval it had been lying beneath a cover-glass in a damp chamber. Another larger, dead Callidina is shown in D (× 250), which contained four unequal but very active Ciliates. The remains of another remarkable specimen is to be seen in E (× 200), which when first observed was a seething mass of eight large Ciliates within the distended body of a Callidina, from which, under the stimulus emanating from the

[1] Mr. Charles Rousselet informs me that the species in question are *Callidina constricta* and *C. vorax*.

lamp, the three specimens seen outside broke away. Fearing that the others would also escape I at once ran a weak solution of osmic acid beneath the cover-glass, and thus prevented their exit. One of the three immature Ciliates which escaped is to be seen more highly magnified in F (× 375). It is fairly typical in shape and development, showing the pointed anterior extremity, about which alone cilia are to be seen, while the body is densely packed with great corpuscles. One of the other specimens has either contracted into a spherical form, or else is seen from a different aspect.

After these immature Ciliates have lived a free life beneath the cover-glass for two or three days they, like the Otostomas to be described in the next section, become developed and altogether altered in appearance. The great globules in their interior gradually disappear and the body assumes a more elongated shape; it becomes marked with coarse longitudinal striations, and along these lines cilia are developed all over the body instead of being present merely about the anterior extremity. The disappearance of the globules is also associated with the development of a rapidly vibrating lip, so that this Ciliate is shown to be a new form of Glaucoma. The characters above referred to are to be seen in G and H (× 375) in each of which also a large rounded nucleus is observable. G was focussed principally on the surface so as to show the longitudinal striations; but in H the cilia all over the surface are better shown, as well as the vibratile lip at the upper border. All intermediate stages between these immature forms filled with large globules which are found within the dead Rotifers, and the developed specimens such as are shown in G and H, have been repeatedly seen.

In some cases eggs in different stages of their development into Rotifers have been found in association with Glaucomas within the dead bodies of parent Callidinas, such as may be seen in Fig. 97, D. I have in my possession also several other photographs showing such combinations. In one of them the body of the dead Callidina contained an egg within which was a fairly developed Rotifer whose jaws were to be distinctly seen, and with it there were four of these active Ciliates. In another, two eggs in an early stage of development were to be seen, together with four active Ciliates. While in a third specimen one normal egg was present in association with six active Glaucomas.

In Fig. 97, A (× 250), a specimen of a doubtful nature is seen, in which the egg had become changed to a light brown colour of just the tint presented by the Ciliates which, as we have seen, are so frequently to be found within the bodies of the dead Callidinas. B (× 250) shows a small Callidina in which considerable remnants of tissues still exist in whose midst, in the

situation usually occupied by an egg, was an Embryo Ciliate full of the usual corpuscles and almost imperceptibly moving within an envelope. C (× 250) represents another specimen of the same kind, only a little larger, in which very slow movements of revolution were also seen, though these movements seem to have stopped while the photograph was being taken, judging from its appearance. In B, on the other hand, the effect of the movement is seen in the lack of definition of the globules contained within the Ciliate. In D we have a different state of things—a normal egg together with a great Ciliate slowly revolving within an envelope being all that is left within the integument of what was originally a Callidina. In neither of these Ciliates were any cilia to be seen, because the organisms were moving within closely fitting envelopes. The movements of the specimen in D were more rapid than those seen in either of the others, so that they had to be stopped with a dilute osmic acid solution before the photograph could be taken.

Were the envelopes within which these creatures were moving the cases of eggs whose substance had been transformed into the Ciliates? Some further evidence bearing upon this question must now be adduced.

In Fig. 98, A (× 250), we have a representation of one of the large-ribbed Callidinas whose normal contents had disappeared, except for the jaws, together with one free Ciliate and one within an egg-case. The egg-case presented some indication of having been ruptured at the lower part of the right end, through which probably the smaller of the two Ciliates had escaped. This supposition is strengthened by the fact that after having taken the previous photograph, and when proceeding to examine the specimen again, the cover-glass was accidentally pressed upon, so as to damage the specimen in the manner shown in B (× 375). The slight pressure had caused the egg-case to open out in precisely the situation where from the previous photograph it seemed possible that it was ruptured, so that it has now given exit to a portion of the squeezed Ciliate. This large specimen was full of great globules, of an unusually large size, and about its anterior extremity, as seen on the right, there were a number of short cilia by means of which it was moving about within the egg-case. The free specimen which had had a larger area (in the body cavity of the Rotifer) in which to move about, had undergone some development, seeing that cilia were present pretty uniformly over its body, while this had also assumed an approximation to its adult shape.

In C (× 60) another Callidina envelope is seen containing six ciliates. They were moving very slowly, and as beneath the same cover-glass there were other specimens that I wished to observe for a time I abstained from bringing their movements

to rest by an osmic acid solution, and photographed them under a low power, in order to show that one of these Ciliates was still within an egg-case. Then, again, in D (× 250) a free egg-case is shown within which there was an active brown Ciliate having the usual appearance except that its contained globules were rather small. As now represented it is in a contracted state after the application of an osmic acid solution.

The differences already pointed out and illustrated in Fig. 96, F, G and H, between the immature and the mature forms of these Glaucomas found in the egg-cases and within the bodies of Callidinas are of great importance in reference to the question of their origin.

The fact that in both these situations it is always the immature form of the Glaucoma which is seen, and that when one of them only exists within the body of a Callidina it is never found to be of smaller bulk than that of one of the Rotifers' eggs, must be considered strongly to point to the conclusion that they have been formed where they have been found, and formed too of the size indicated. Had minute specimens of the Ciliate obtained access to the body of the Rotifer and even into its eggs, then they would be seen in these situations as minute forms, and of sizes intermediate between them and that of the full size actually met with. Very small forms, however, are never seen in the first instance, and it is only when their numbers increase by the occurrence of processes of fission that the sizes met with fall appreciably below the average. It cannot be considered, therefore, that the immature forms found within the bodies of the Rotifers, and even within their eggs, have arisen from minute immature forms which have in any way obtained access to the bodies of the Rotifers and their eggs.

And if developed forms—such as are to be seen outside the bodies of the Rotifers—with their more elongated bodies, longitudinal striations, and general development of cilia had been able to force their way into such Callidinas as are seen in Fig. 97, B and C, they would, of course, be found presenting these fully-developed characteristics—which is never the case. They appear first as spherical or pyriform creatures, having their bodies filled with great corpuscles, and with a few small cilia only about their pointed anterior extremities; or else in the situation usually occupied by an egg, and revolving within a hyaline envelope which is in no way different from the egg-case of one of these Rotifers.

All this constitutes a mass of evidence of the greatest cogency (little short of absolute proof) in favour of the view that some of the Glaucomas found in the bodies of dead Callidinas have been produced by a transformation of the substance of the eggs of such Callidinas, and that those first produced are prone to multiply by

fission while devouring all that is to be found within the integuments of the parent organism save its horny jaws. But precisely the evidence which is lacking here—namely, the actual observation of the different stages in the development of the Ciliates from the eggs of these Callidinas—will be found to be forthcoming in the next section, in regard to the transformation of the eggs of Hydatinæ into Ciliated Infusoria.

IX. ON THE TRANSFORMATION IN THE COURSE OF THREE OR FOUR DAYS OF THE ENTIRE CONTENTS OF THE EGG OF HYDATINA SENTA INTO A LARGE CILIATED INFUSORIUM BELONGING TO THE GENUS OTOSTOMA.

This remarkable transformation has been already in part described in Section III. (*d*), p. 45; and what I have there said concerning it, and the means by which it has been brought about, on pp. 45, 47-49, 53 and 54, has been since, in all respects, confirmed.

I have, in fact, during the last five months devoted much time to the study of this particular transformation, and have consequently extended considerably my knowledge in regard to it, in several directions. I have devoted special attention to it, because, unlike so many of these heterogenetic changes, the transformation in question can, with a proper supply of *freshly-laid eggs*, be induced almost at will. This consideration, together with the extremely important nature of the change, led me to try and make the fact of this transformation and the methods by which it can be brought about known to the world of science, through the medium of the Royal Society, and one or other of the European Academies of Science. But in none of these Societies were the officials sufficiently open-minded and receptive to receive and discuss observations so much at variance with generally accepted views. By all of them my communication was consigned, unread, to their Archives. The unanimity of this procedure on the part of these learned Societies was a striking revelation of the persistence, even to the present day, of that kind of intolerance of new truths which history has revealed to us as existing in the Middle Ages. It only remains for me, therefore, to submit my methods and their results to a wider audience, in the hope that some day they may arouse the attention of workers who are more open-minded, and more ready to appreciate the evidence in favour of new facts, even though they should be altogether at variance with their preconceptions.

Only one species of this genus *Otostoma* has hitherto been known, and that was a form originally found and described in India by H. J. Carter. Other specimens of this species are stated

by Saville Kent[1] to have been found in Devonshire in 1880, by the present Hunterian Professor of the College of Surgeons, Charles Stewart. This, I believe, is the only previous record of the existence of such an Infusorium (or of any other representative of the genus) in this country, so that it can only be regarded as a very rare species.

All that I have said on the pages above indicated as to the means by which this Ciliate is to be obtained from a fresh Hydatina egg remains good. The points that I would especially emphasise, however, are these:—

(1) Use small earthenware, half-ounce pots with closely fitting covers, and very nearly fill them with tap water, so as to leave only a small air space when the cover is applied.

(2) Do not use eggs which have been laid in the very foul water immediately draining away from some manure heap, where Hydatinæ often swarm in association with a film of Euglenæ. Such a fluid is so crowded with Bacteria that eggs cannot be obtained from it, after an interval, without being enveloped and more or less obscured by a scum of Bacteria.

(3) My best results have been obtained by taking some surface mud covered with a layer of Euglenæ, or of Euglenæ mixed with Chlamydomonads, placing it at the bottom of a suitable glass vessel and pouring water over it to the depth of four or five inches. If water only to the depth of one or two inches is poured over the mud the Euglenæ will soon rise and form a thick scum on the surface of the fluid, which is not desirable. But with the greater depth of water I have mentioned, especially if for twenty-four hours or so the vessel is kept in a dim light, very few of the Euglenæ will rise to the surface. Where there is a scum of Euglenæ the Hydatinæ will lay their eggs, in the main, in patches among these Euglenæ. Such patches may be easily taken up with a knife and transferred to the pot, but then they are obscured by the Euglenæ, and always float on the surface. On the other hand, where there is little or no scum or foreign bodies on the surface of the fluid the Hydatinæ will lay their eggs in the main on the side of the vessel at and near the line where it comes into contact with the surface of the fluid. From this situation it is easy to scrape up batches of 30 to 40 of the Hydatinæ eggs, and to push them off from the scalpel in a single mass into the water contained in the pots. Most of such masses of eggs will sink, and I have found that a larger proportion of transformations takes place in these eggs lying at the bottom of the pot, than in those which remain at the surface of the fluid.

(4) The sooner, however, that the eggs are gathered after they have been laid the larger will be the proportion of them which undergo this transformation into Otostomata. The reason of this is due to the fact that the normal development of the egg commences very soon after it has been laid; so that in any ordinary batch of Hydatina eggs taken up for examination, eggs in all stages of development will be found, associated with a few others in which there is no appreciable indication of any developmental changes. These, or some of them, are the only eggs in which the transformation in question, under the abnormal experimental conditions, is capable of being brought about. Large numbers of such eggs can only be obtained where we have to do with large numbers of Rotifers, and the most certain means of obtaining the eggs fresh is this: Remove all eggs that are to be seen on the side of the vessel near the surface of the fluid, and then, six or twelve hours later, gather all the eggs that can be found, and transfer them to the pots.

[1] "Manual of the Infusoria," 1881, vol. ii., p. 500.

(5) The temperatures most favourable to the change lie, I think, between 60° and 65° F. My most successful results have been obtained when the pots have been left in a cupboard at such temperatures; on the other hand, I have had very many failures when the pots have been kept at a constant temperature in or upon an incubator, so that I have now given up this procedure.

(6) It is well, at the same time, to examine one or two of these batches of Rotifers' eggs under the microscope, so as to make sure that no Otostomas are to be found among them; and also to place some of them in a watch-glass exposed to light, so that its contents may be examined from time to time during the next three or four days. Under the latter circumstances I have never found Otostomas either free or encysted; while in the course of three to four days each of the batches of eggs taken from the bottom of the dark pot may be found to contain from 3 to 10 of these great Ciliates, either still revolving within the egg-cases or breaking out therefrom.

Acting in the manner above indicated we may sow Rotifers' eggs, and within the brief space of three or four days we may find that many of them have given birth to great Ciliates. Postponing for the moment some description of the mode in which this astounding transformation is so rapidly brought about, I have now something to say as to other conditions under which, as I have subsequently found, these great Otostomas will make their appearance in association with Hydatina eggs.

I have ascertained, for instance, that it is not absolutely necessary to cut off every vestige of light rays by enclosing the eggs within a stone pot. If they are put into a small covered glass vessel similarly full of water, with a similarly small air-space above, and then merely cut off from ordinary day-light by placing them in a dark cupboard, the same kind of transformative changes will occur, only they will be brought about much more slowly. I have found it take from eight to ten days to lead to the production of fully-formed Otostomas within some of the eggs in the glass vessel; while other eggs from the same source, contained in a stone pot, standing by the side of the glass vessel, will go through similar transformations in the course of three or four days. This makes me think, therefore, that the stone pot cuts off some invisible rays of light which may be capable (like Röntgen rays) of passing through the wooden door of the cupboard, and that the cutting off of such invisible rays favours and hastens the process of transformation.[1]

Then again I have ascertained that occasionally, when a given stock of Hydatinæ and Euglenæ has been kept within a room in a comparatively dim light, and during part of the time under a bell-jar so as to protect it from dust, some of the Hydatina eggs will, at periods varying from twelve to eighteen days, be found to undergo similar transformations into great Otostomata. It is not only that these great Ciliates are

[1] In pp. 55-58, where I have spoken of "Röntgen rays," I ought only to have spoken of "invisible light rays," as above; as there seems to be no positive evidence that Röntgen rays enter into the constitution of solar rays.

occasionally to be found among the eggs in the stock vessels, when not a trace of one could be discovered during earlier days, but that among eggs taken from such stock vessels, after they have been kept for the periods mentioned, I have several times discovered eggs exhibiting different stages of the transformation such as I am now about to describe and illustrate.[1] Many of the Otostomas found under these conditions have grown considerably, so as to be very much larger than those taken from the dark pots.

The changes in question seem to occur with equal frequency in the large and the small eggs laid by Hydatinæ. It needs only a comparatively brief acquaintance with these creatures to recognise that their eggs vary a good deal in size. It is commonly believed, and I think with truth, that the smallest of the eggs give birth to the small male Hydatinæ. But between such small eggs and the large eggs there is no abrupt demarcation. There are plenty of eggs also of intermediate dimensions, and these give birth to female Hydatinæ, although to specimens falling short of the full size.

I shall show some of the stages of these transformative changes leading to the production of Otostomas, first of all in large eggs, and subsequently in the small specimens.

In Pl. x., Fig. 99, all the components of which are magnified 250 diameters, the early changes leading to the production of the great Ciliate are represented. A shows one of the large eggs recently laid, and presenting the usual evenly granular condition. In B we may see the first indications of the heterogenetic change occurring at the periphery, especially below and to the right. Such changes, further advanced, are shown in C; and further advanced still in D, where the whole egg substance is evidently becoming converted into a mass of hyaline vesicles, intermixed with granules and containing granules in their interior, *an appearance which is never seen in Hydatina eggs undergoing their normal development.*

The amount of granules within and between the vesicles varies extremely in different specimens. Sometimes, as in Fig. 100, A (× 250), there are comparatively few in either situation. The egg is then pale, and the individual vesicles being very distinct may be seen to vary a good deal in size. At other times the granules in both situations are much more abundant, as in B (× 250), and it is only at the periphery of the egg that the vesicles can be distinctly seen. As development proceeds, the granules tend to increase again, so as sometimes almost completely to obscure the vesicular structure. Such a

1 It is to be expected, therefore, that occasionally such changes may take place in the natural habitats of the Hydatinæ, under certain conditions.

condition is shown in C, in which the contents of the egg was still motionless, and in the stage just anterior to the development of cilia. In D, on the contrary, cilia had just become developed, and the embryo Ciliate was beginning to revolve in a slow and almost imperceptible manner, so that a weak osmic acid solution had to be run in beneath the cover-glass before the photograph could be taken. No cilia are to be made out, because within the egg-case—the outline of which is distinctly to be seen to the left and below—the Ciliate is still enveloped by a closely-applied hyaline membrane.

The existence of this hyaline envelope, which, though itself invisible, by its presence conceals the cilia, is still better indicated in Fig. 101, A (× 250), representing a specimen formerly revolving, which was killed by a strong osmic acid solution and then preserved in a mixture of glycerine and water. It will be observed that the egg-case has been broken, and that the embryo Ciliate, stained by the osmic acid, has shrunk to a notable extent, together with its hyaline envelope, so that no cilia are visible. This specimen contrasts not ably withB (× 250), in which we have the representation of a Ciliate that has just been forced out both from its egg-case and from its hyaline envelope, and is seen to be completely fringed with cilia. It was killed with a solution of formalin and this has, as usual, dissolved many of the globules and rendered the whole organism more transparent. It generally reveals the large nucleus also as an elongated somewhat spindle-shaped body. A contractile vesicle is not commonly to be seen at this early stage in the life of the organism, so that the clear body here shown is, I think, the nucleus seen endwise. C (× 250) shows a free swimming specimen just out from the egg-case, which was killed with a weak solution of iodine. It presents the common appearance of these organisms as they escape, where the body is densely packed with brownish globules, which are apparently modified representatives of the original vesicles so abundantly present in the earlier stages of the transformation.[1] Sometimes, however, such globules are scarce or almost absent, their place being taken by mere granules, and then the large elongated nucleus is often visible without the aid of reagents, as in the small specimen shown in Fig. 102, A.

Whether the organisms have the body densely packed with brownish coloured globules as in Fig. 101, C, or with mere granules more like B, on escaping from the egg-case, I have found to depend in the main upon the length of time that they have been revolving within the egg-case previous to their escape. If the

[1] These more opaque globules of the developed organism often assume their original vesicular appearance when the specimen is mounted in a mixture of glycerine and water.

pots are opened soon after their complete formation the Ciliates are found to be densely packed with globules; but if they are not opened for two or three days after the time in which they are usually formed, the Ciliates are found still revolving within their egg-cases, but containing only granules in their interior. It would appear that after the complete formation of the Ciliates they go on slowly revolving within the egg-cases, so that the globules are probably in part used up as food products during the two or three days in which they are doing this work, while obtaining no food from without. As a rule it is not till the pots are opened that the Ciliates under the stimulus of light (and especially the concentrated light of the microscope lamp), begin to move more rapidly within the egg-cases, and succeed in effecting their escape. Once free, they dart away with the most rapid movements, associated often with rotations of the body on its longitudinal axis.

Occasionally while still within the egg-case the embryo Ciliate divides into two, and one of the products of such a division is shown in Fig. 102, A ($\times$ 250), in which a large elongated ovoid nucleus is partly revealed. In C ($\times$ 250) another specimen is shown, in which segmentation into four has taken place, one of the segments being at a deeper level and therefore hidden. This specimen has been forced out from its egg-case by the pressure of the cover-glass, though the segments were still contained, and were revolving, within the invisible hyaline envelope. One of the segments into four of another organism, from a pot which was first opened on the seventh day, is shown in B, in its free swimming state. It is rather more elongated than usual and its body contained blackish granules, rather than the brownish globules more commonly to be seen. In D a free undivided specimen is shown which had probably been long revolving within its egg-case, from which it had, however, only recently escaped. It is altogether unusually free both from globules and from granules, and therefore serves well to show the fine longitudinal striations of the body along which the rows of cilia are found; and also the small characteristic ear-like mouth the existence of which caused H. J. Carter, who first discovered the type of this genus in India, to give to the genus the name *Otostoma*. His type specimen was found within the decaying filaments of a species of Nitella. The specimens derived from the transformation of the eggs of Hydatinæ evidently belong to the same genus, as may be seen from its description and that of the species discovered by Carter, as given in Saville Kent's "Manual of the Infusoria" (vol. ii., p. 500, Pl. xxii.). In the specimens as they come from the Hydatina egg-shell either no contractile vesicle, or only one, is at first seen, but in regard to the approximate size and shape of the organism, its longitudinal striations,

the character of the nucleus, together with the situation and shape of the ear-like mouth, there is the closest accord between it and the form to be found at times within decaying Nitella cells.

That similar transformations to those above described occur within the small male eggs may be seen by the photographs composing Fig. 103, all of which have been taken at a magnification of 250 diameters. I only show a few of the stages, omitting other intermediate states of which I have representations, as the changes are so obviously similar to those occurring in the larger eggs. A shows a newly-laid specimen of one of these small eggs. B shows one of the early stages of the transformation in which characteristic vesicles are beginning to form, such as are to be seen well developed in C. In D we have a representation of what was a very slowly revolving Ciliate within one of these small egg-cases, and still within its hyaline envelope, so that no cilia are visible. While in E we have a typical but small Otostoma formed from one of these small eggs, which has just escaped from its egg-case.

My experience, since the discovery of this remarkable transformation of the substance of the Hydatina egg, has been such as to cause me no little surprise, owing to the incredulity and scepticism I have had to encounter during my efforts to make this discovery known to biologists generally, in the usual manner, through the medium of the Royal Society and one or other of the European Academies of Science. It will be well for me, therefore, to add some additional elements of proof to those which I have already furnished.

To any one who has worked at the subject, as I have done, for some months and has seen hundreds of these great Ciliates, when taken from the pots, either in or emerging from the Hydatina egg-cases, there could be no room for doubt as to the reality of this transformation. Those, however, who have been told of these changes, even when they have seen the photographs and some of the specimens, have in many cases remained profoundly incredulous. Thus one eminent Professor of Zoology, in reply to an invitation to come and see some of the living specimens, wrote as follows: "Nothing short of the demonstration by actual observation, continuous and unbroken, of the links connecting the egg (whether partially developed or not) with the Infusorian, would convince me of the accuracy of your conclusions." This was a requirement not likely to be satisfied, seeing that the change, as I then knew it, took place only when light of all kind was excluded, and was stopped as soon as light was admitted. I could only reply, therefore, that I trusted to find others who

would be more amenable to reason. Another former Professor of Zoology was so sure of his own ability to gauge the potencies of natural phenomena that he refused even to look at the specimens or photographs, close at hand, in illustration of this remarkable transformation.

Yet one may almost see the eggs laid by the Rotifers busy in their midst. For the eggs are large enough to be detected with the naked eye and can be most easily recognised with the aid of a pocket-lens. When they have accumulated sufficiently at the side of the vessel, masses of 20 to 30 or more may be taken up with a knife, and transferred to a drop of water on a microscope slip. We may then see that the little white spheres are all Hydatina eggs, either newly laid or in different stages of development, and that there are no Ciliates among them either free or encysted; we may place these masses of eggs in the pots as I have directed, and in three or four days we may open the pots, and find in each batch of eggs from 3 to 10 of the great Ciliates revolving within as many of the egg-cases. And if we open the pots earlier we may see the intermediate stages in the transformation as they are shown by my photographs.

It seems difficult, even allowing for a large amount of natural incredulity at first experienced, to put more than one interpretation upon such facts. The unbiassed exercise of reason upon the evidence adduced ought to make it clear.

I have, however, other proofs which, added to what I have already set forth, should suffice to bring conviction even to the most sceptical. To one of these further proofs I will now direct the reader's attention.

During the last three or four years I have often observed that when Hydatinæ have been kept indoors for ten or more days, they lay eggs very much darker in colour than usual—so that they have an almost black appearance, owing to some slight alteration in the nature of the granules entering into their formation. About the same time "resting eggs" begin to appear, and also male Hydatinæ.

I am aware that some authorities regard it as settled that the Hydatina "resting eggs," are eggs which have been laid by fecundated females. Although I am not prepared to traverse this view, my experience leads me to think that "resting eggs" are often found in abundance before male Hydatinæ make their appearance; and I am inclined to think that the formation of "resting eggs" is intimately related to that change in the constitution of the egg to which I have just referred as occurring almost invariably when they have been kept long in confinement. All stages may, indeed, often be seen between the ordinary eggs full of blackish granules that are to be found in these media (both free and within the bodies of the Hydatinæ), and the similar

black granule eggs to which a thick spinous envelope is added—which may also be seen both free and within the bodies of the parent Hydatinæ.

When masses of eggs of this kind, which for brevity we may speak of as black granule eggs, are taken and batches of them are placed in the pots in the usual way, those that are freshly laid undergo the transformation into Otostomata, in the manner I have described, and the Ciliates to which they give rise are also filled with black granules during their formation and when they emerge from the egg-cases. Thus Fig. 104, A (× 125) shows one of these black granule eggs; while B (× 125) shows another that had gone on to the formation of a Ciliate densely packed with black granules. This was now segmented into two, and, before the application of a formalin solution, each segment was very slowly moving within the egg-case. Although no large corpuscles are to be seen in the photograph, yet examination of the specimen with the microscope showed that they were present though hidden by the abundance of granules. Of course no such complete segmentation as this, with slow rotations of the separate parts, is ever to be seen during the ordinary development of a Hydatina egg.

In C (× 250) we have a representation of one of the great Otostomas just after it had emerged from the case of one of these black granule eggs. The granules in it do not completely hide the globules, and the appearance of these within this ciliated organism is strikingly like the vesicles which enter so largely into the composition of the egg-mass during earlier periods of the transformative process. D (× 250) shows one of the products of fission of a Ciliate formed from such an egg. In it the granules are again so abundant as almost completely to hide the usual great globules which it also contains. In E (× 80) there is shown, at a much lower magnification, a group of Otostomas produced from black granule eggs, situated among a mass of egg-cases from which Hydatinæ had escaped. Most of the Ciliates were seen revolving within their egg-cases, though others had escaped and, before they were killed with a weak formalin solution, were slowly moving among the empty cases. These empty egg-cases had been left by Rotifers which had already commenced their development before having been placed in the experimental pot. As soon as their development is completed they commonly make their way out, and do not wait for the stimulus of the light as is commonly the case with the newly-developed Otostomas.

These observations are surely very conclusive. Directly the eggs vary from their usual condition and become filled with black granules we find a concomitant variation in the Ciliates issuing

therefrom—which is, of course, only to be expected if the Ciliate is formed from the substance of the egg.

But even this does not exhaust the evidence telling in favour of the reality of this transformation of the whole substance of the newly-laid Hydatina egg, when placed under certain abnormal conditions, into a great Ciliate belonging to the genus Otostoma. There is the additional fact that I have seen eggs, in the intermediate or vesicular stage of this transformation within the body of a dead Hydatina; and the further fact that I have seen a fully-developed active Otostoma within the dead body of another Hydatina. These observations have been made under circumstances which I will now detail.

When one of the experimental pots was opened at the end of the second day in order to obtain specimens of the eggs in the intermediate vesicular stage of the transformation, after having taken out all the batches of Hydatina eggs that were visible at the bottom of the pot, I noticed about a dozen separate bodies just the size of Hydatinæ. These were taken up with a small pipette, and on examination I found that they were really dead Hydatinæ, in different stages of decay, but that nearly all of them contained two or three eggs each—in different developmental conditions though all were about equal in size. In one, for instance, there were the three eggs which are shown in Fig. 105, A (× 150). The upper one is a black granule egg apparently in a mature condition; another may be in an early stage of normal development; while the light egg contained a moving and nearly fully-developed Hydatina. The contents of another of these dead Rotifers is shown in B (× 200). Here also there were three eggs, the contents of one of them had almost disappeared, but the other two undoubtedly represented different stages in the transformation of the eggs into Otostomas. In the one in which the changes were most advanced, the vesicular condition was most distinct. This is a condition invariably met with where the contents of the eggs are being transformed into Otostomata, though nothing like it is, as I have said, to be found during the normal development of the Hydatina egg. I found eggs in a very similar condition in two others of this group of dead Rotifers; but since then, though I have many times searched for them, I have failed to find eggs developing either normally or abnormally within dead Hydatinæ. This is rather remarkable, and in great contrast with what obtains among the dead Callidinas from the Luchon lichen.

Some explanation of this difference may possibly be found in the different media from which these two kinds of Rotifers are taken. The Hydatinæ are commonly found in more or less foul waters swarming with Bacteria, and, apparently as a consequence, after their death they are found to rot rapidly and

disappear, leaving in the course of two or three days a mere heap of granular *débris*, the nature of which is indicated merely by the presence of the jaws, which resist decay for a much longer period. With the Callidinæ, however, even after the lichen has been soaking in distilled water for six or seven days, the medium is different. Although it may and does commonly swarm with Bacilli, they are not putrefactive organisms and the bodies of these dead Rotifers will remain in this fluid for many days without undergoing disintegration, and during this period any eggs that they may contain grow and develop. That eggs which are small at the time of the death of the Callidinas grow so as to attain their full size is evidenced by the fact that though it is rare to see these living Rotifers bearing more than one fully-developed egg at a time, it is quite common, as I have shown, to find within them, when dead, from two to four eggs of equal size and in different stages of development, as shown in Fig. 95. It is common also to find from one to eight Glaucomas within the bodies of these dead Callidinas, presumably developed from such eggs.

These facts seem to point to the conclusion that the particular Hydatinæ found by me in this one experimental pot may have come from some medium purer than usual, and must have died nearly simultaneously from some unknown reason. They consequently did not rot so quickly and there was time for the eggs to grow and undergo this or that stage of development. Once afterwards I found some Hydatinæ which had been placed in a watch-glass about thirty-six hours previously, dead and containing also two or three equal-sized eggs. What had caused them to die in this way I could not discover, and in various attempts which have been made since I have never succeeded in annulling the life of the Hydatinæ without destroying the vitality of their eggs.

Now, as to the finding of a living Otostoma within the body of a dead Hydatina: this was seen under the following circumstances. I had a stock of Rotifers in confinement for twelve days, and the vessel in which they were contained had been standing on the mantelpiece of my study for the greater part of that time. Much evaporation of the fluid had taken place, and no distilled water had been added to compensate for the loss. On examination I found mixed with the Euglenæ on the surface numerous amorphous saline concretions resulting from the evaporation; also "resting eggs," and many ordinary black granule eggs, together with Hydatinæ containing eggs of each variety. I took up an isolated batch of these eggs in association with some Euglenæ, two or three of the Rotifers, and some of the saline concretions, and placed them in a drop of distilled water under a

cover-glass. On examining this specimen with the microscope I found that several of the eggs seemed to be freshly laid, so I resolved to try whether a transformation of any of these eggs could be brought about under the cover-glass—which was prevented from pressing upon the eggs by the presence of the saline concretions. I accordingly placed a shallow layer of water on the wrong side of one of a pair of 'tinting saucers,'[1] placed the microscope slip within it, and over it the other saucer right side downwards: the two together making an excellent damp chamber, with a very limited air space, in which the specimen would be cut off from light rays of all kinds.

When the specimen was looked at after the expiration of three days I found that about one-third of the water beneath the cover-glass had evaporated, but on examination with the microscope I found three great Otostomas revolving within unruptured egg-cases; and within the body of a dead Hydatina, which was only partially decayed, so that its integument was quite whole and remains of its internal organs were distinct, was a great Otostoma densely filled with black granules, free, and moving about in a very active manner. The whole body of the Rotifer when thus seen was visible, but unfortunately after running a weak osmic acid solution under the cover-glass the Rotifer became partly covered by some of the saline concretions, and after various ineffectual attempts to dislodge it I was obliged to be content with photographing it as it is seen in Fig. 105, C ($\times$ 150). No other free Otostoma was found beneath this cover-glass. There were only the three within egg-cases, and the one free specimen within the body of the dead Hydatina.

This was a very encouraging result, and I have several times tried to get a repetition of it, but as yet ineffectually. At first my failures were due to putting the experimental saucers within an incubator at a temperature of 62° F., but this equal heat day and night did not answer, as it almost always led to the evaporation of the water beneath the cover-glass before the time came for examining the specimens. Then, again, I used eggs from stocks which had not been long in confinement; and when, after several failures, I realised that in attempting to obtain this transformation under such difficult conditions (that is in a minute quantity of water beneath a cover-glass) I ought, as at first, to use eggs from Rotifers which had been long in confinement, my supplies of Hydatinæ failed me. In future attempts, with better supplies and warmer weather, I hope to be more successful. Then, again, the egg-bearing Rotifers ought to be placed in a drop of tap water rather than in one of distilled water. It is true that

[1] Made of white earthenware, about four inches in diameter, and obtainable from Messrs. Swift and Son, of 81, Tottenham Court Road.

distilled water was used previously, but this defect was then corrected by the presence of the saline concretions with the Rotifers and their eggs. I have, however, since ascertained that this transformation of the Hydatina egg into the Otostoma will not take place in pots containing distilled rather than tap water—nor will the normal development of the egg occur under such conditions.

* * * * * *

Since the foregoing matter was written I have found what I have never seen before in the various batches of Euglenæ examined during the last five years—that is, multitudes of Otostomas living and multiplying freely in a fresh Euglena pellicle which was fully exposed to ordinary daylight. I have moreover been able to keep them for four weeks under confinement, till hundreds of them had become encysted, and have thus been able to ascertain that in their encysted condition they are wholly unlike anything that is to be seen during the stages of their origin from the Hydatina eggs. This has happened under the following conditions.

The original site from which I obtained supplies of Hydatinæ having failed me owing to building operations and the laying down of a sewer in the road, I had to look elsewhere for further supplies and ultimately took from a different ditch, into which water also ran from a farm-yard, a thin layer of surface mud coated with Euglenæ. This was put, in the ordinary way, into a glass vessel; water to the depth of about four inches was poured over it, and it was left exposed to light. A Euglena pellicle formed in due course, but I did not examine any of it till the sixth day, and then my surprise was great to find that it was absolutely swarming with Otostomas. With them were multitudes of small Vorticellæ and of Chilodons, and also many Zooglœa masses, together with Hydatinæ and their eggs.

Some of the Otostomas were very large, having nearly twice the diameter of those which emerge from the Hydatina egg-cases; though others were comparatively small and evidently products of fission into two or into four—many instances in which this process was taking place having been seen. Fig. 106, A (× 125) shows one of these moderately large Otostomas, only magnified half as many diameters as the specimen represented in Fig. 103, E. Then, again, the specimens varied very much in appearance. Some of them were filled with the great globules such as are found in the Otostomas which originate from the Hydatina eggs; though very many of them had no such globules, and merely contained more or less granular contents such as I had previously found in specimens that had been living a free life even for a few days.

Although there were in this same pellicle multitudes of Hydatinæ and of their eggs, yet in the portions of the pellicle that I examined I found no specimens in which the eggs were being transformed into Otostomas—that is, no specimens of the eggs in the characteristic vesicular stages. I came to the conclusion, therefore, that in this site the Otostomas, whenever and however they may have originated, were evidently living and multiplying in great numbers after the manner of Ciliates generally.

I therefore took portions of the pellicle up with a section lifter, and transferred them to a small beaker containing about four ounces of ordinary tap water, with the object of examining the Otostomas ultimately in their encysted condition. The beaker was left on the mantelpiece covered by a glass shade, and from time to time during the first two weeks I examined its contents with a pocket lens, and could see large numbers of Otostomata, together with Hydatinæ, swimming about all through the fluid. Now, after four weeks, very few of them are to be seen in the fluid, and the remains of the portions of pellicle have all dropped to the bottom of the vessel. On taking up some of these fragments with a small pipette and examining them under the microscope, I found large numbers of the Otostomas encysted (varying a good deal in size), and with them also multitudes of the very much smaller encysted Vorticellæ and Chilodons, but no Hydatina eggs. Some of these encysted Otostomas are to be seen in Fig. 106, B (× 60), as they appear under a low power.

Examination of such encysted Otostomas with a higher power shows that they are nearly all quite motionless, though, after their exposure to the light of the lamp for a few minutes, some of them begin to show very slow movements of rotation—so slow as to be scarcely perceptible.[1] Their cysts are smooth and colourless, but very much thicker than the Hydatina egg-cases. They are also often composed of two or three distinct laminæ, and a slight space generally exists between the cyst and the contained organism. As a rule no nucleus or contractile vesicle is to be seen; the substance of the encysted Ciliate is throughout closely packed with granules and very minute corpuscles, such as are shown in Fig. 106, C (× 250)—which is the representation of a small specimen rather less opaque than usual and in addition slightly compressed. The more ordinary appearance of these encysted Otostomas is represented in Fig. 107 (× 250), the surface of which was focussed in order to show that its internal texture is similar to that more plainly seen in C. These encysted Otostomas now exist in the greatest abundance at the bottom of

[1] The application of a dilute iodine solution, however, causes them to rotate much more rapidly.

this small vessel, in which formerly hundreds of them were to be seen, with a lens, swimming about all through the fluid.

How long the Ciliates will remain in this encysted condition it is of course impossible for me to say; but their discovery in this state enables me to round up my evidence in a very satisfactory manner by showing how totally unlike the Otostomas when thus encysted are to the Otostomas that have originated from the metamorphosis of Hydatina eggs, and which may be seen emerging from their egg-cases. A comparison of Figs. 99 and 100, with Fig. 106, C and D, should make this difference plain even to the most sceptical.

As to the reality of this metamorphosis there is, in fact, absolutely no room for doubt. The only two conceivable sources of error have been proved to be unreal and untenable, as was set forth in the "Note" that was addressed in vain to the learned Societies, and which I have thought it well to reproduce as an Appendix.[1]

X. ON THE TRANSFORMATION OF THE SUBSTANCE OF THE EGGS OF A TARDIGRADE (MACROBIOTUS) INTO CILIATED INFUSORIA.

The Tardigrades on which the following observations have been made have all belonged to the genus Macrobiotus, and on reference to the plates and descriptions given by Doyère in his celebrated "Mémoir sur les Tardigrades"[2] it is clear that they have all been specimens of *M. Oberhaeuser*. These curious creatures were first observed and described by Eichhorn in 1767, and named by him 'Wasserbären' or Water-bears; while later on they were termed 'Sloths' by Spallanzani, who devoted much attention to their tenacity of life and power of recovery after long periods of desiccation in tufts of moss and lichen.

The specimens that I have had under observation have been found in association with the great Amœbæ and the Rotifers obtained from portions of the yellow lichen *Parmelia parietina* —brought from Bagnières de Luchon seven months previously— after they had been soaked in distilled water from seven to ten days. The fragments of lichen were allowed to soak for this long period (the weather being cold) because in this way, as I have previously indicated, many more of the specimens of Amœbæ, Rotifers, and 'Sloths' dropped away from the lichen and were obtainable than would have been the case if the soaking had only been for the few hours needful to enable these and other organisms existing in the lichen to resume their active life.

A drop of the fluid left after this soaking, together with some

[1] See Appendix II., p. xi.

[2] *Ann. des Sciences Naturelles* (*Zool.*), 1840, p. 269.

of the sediment, was transferred by a tiny pipette to a microscope slip, and on each side was placed a minute fragment of one of the thinnest cover-glasses, so as to protect the organisms from pressure and allow them to move about after a cover-glass has been applied. Every portion of this drop was then thoroughly scrutinised under the microscope, with the result that occasionally one, two, or even three specimens of Macrobiotus would be found —young or old, living or dead.

They are of a red-brown colour, owing to the presence of much pigment of that tint in different parts of the body, but especially in a broad band of pigment cells lining the inner side of the integument along the middle and lateral regions of the back. This superabundance of pigment is one of the distinguishing features of *M. Oberhaeuser.* It is more plentiful in some individuals than in others, and especially in old specimens. Many of them contained great black-looking eggs, varying from two to five in number, such as are shown in Pl. xi., Figs. 108, B., 109, A, 110, A. The curiously irregular shape of these eggs at times may be seen in Fig. 108, A (× 150), which represents a rather large specimen seen from above, and containing several eggs very much lighter and less opaque than usual. It is possible that this large specimen may have been slightly compressed, so that the diminished opacity and irregularity in shape of the eggs may have been in part due to this.

My attention was specially directed to these creatures owing to the following circumstance. I was at the time studying the changes in the eggs of Callidinas, previously described. I placed, on March 18 last, a slip containing specimens of these Rotifers mounted in the manner I have described, in a damp chamber on the mantelpiece of my study. Among them was found a young dead Macrobiotus, containing two eggs smaller than, though otherwise similar to, those shown in Fig. 108, B. The specimen was examined every two or three days, and a little more distilled water was added to that beneath the covering glass if it had begun to evaporate. The single Macrobiotus existing in this drop of fluid was seen on March 22 to be seemingly unaltered, but no photograph was taken as I was specially watching other objects beneath the same cover-glass. It was not again examined till March 25, when to my great surprise I found within the integuments of the 'Sloth' eight very slowly-moving Ciliates, filled with red-brown corpuscles, together with five pale, merely granular, spherical masses. The movements of the Ciliates were so slow that I took the photograph represented in Fig. 95, C (× 150), before the application of a solution of osmic acid. The specimen is thus shown much as I saw it, except for the blurring caused by the slow movements. Nothing of the animal was left within the integument save the pharynx and the suction bulb—

not even the pigmentary lining. The integument itself seemed whole and entire, so that this astonishing transformation, which had taken place within the space of three days, greatly piqued my curiosity.

Subsequently, therefore, I sought for specimens of Macrobiotus containing eggs, in drops of the fluid and sediment mounted in the way I have mentioned, and, if found, the specimen was sometimes photographed at once and kept as before in a damp chamber—that is, in a shallow glass vessel containing a thin stratum of water which was covered with a plate of glass. The specimens were examined afterwards only rarely and briefly, as I feared the possibly harmful influence of the light from the microscope lamp.

Meanwhile, dead specimens of Macrobiotus were from time to time encountered, which, when they first came under observation, already contained Ciliates. One of these specimens seemed of great importance. It is that shown in D (× 150), in which, before the application of a solution of osmic acid, two great Ciliates full of brown corpuscles were revolving within hyaline envelopes, so that the cilia themselves were invisible. The integument of the Macrobiotus was again intact, and here were two comparatively huge spherical Ciliates and little else recognisable save certain masses of pigment along the back. The osmic acid caused their shape to be distorted, and caused the one on the left slightly to break through its envelope. It seems possible that two Ciliates may have taken origin from two eggs; that they devoured the remaining contents of the Macrobiotus without undergoing fission; and that they then began to encyst themselves. In the light of what follows this seems the most probable interpretation.

Another of the specimens found, in which the contained Ciliates were of very unequal size, is shown in E (× 150). It is shown partly on account of this inequality and partly because the integument, apparently intact, and the outlines of the Macrobiotus are so distinctly shown.

On April 20, a dead Macrobiotus containing four closely approximate and rather irregularly shaped eggs was found in a drop of the water and sediment just mounted for examination. The specimen was not photographed at the time, but it was very much like that shown in Fig. 109, A (× 115).[1] It was associated with other organisms which I desired to keep under observation for a time, so that the microscope slip containing it was placed, as usual, in one of the before-mentioned damp chambers. On

[1] This specimen was intended to have been photographed with an enlargement of 150 diameters, but by mistake a No. 6 instead of No. 8 compensating eye-piece was used—hence its smaller size.

April 24 I found the Macrobiotus in the altered condition shown in B (× 150). Its four eggs were no longer closely approximated, so far as the first and second were concerned; each of them had become more or less completely spherical; and there were now distinct indications within of the formation of globules. Recognising its condition I made only a hasty examination, and abstained from taking a photograph at a higher power than 150 diameters, in order to avoid the chance of interfering, by a more prolonged exposure to the light of the lamp, with the progress of changes that seemed to be taking place in the eggs. I much regretted this at the time, especially because of the appearance of the anterior of the four eggs, in which the formation of corpuscles, such as are found in the Ciliates, appeared to be going on, though in their ordinary condition the eggs of the 'Sloth' are simply granular, as shown in Fig. 108, A. On examination after twenty-four hours, I again found that a complete metamorphosis had taken place; the four eggs were gone and with them all that was within the integument of the Macrobiotus, including the whole of its scattered pigmentary lining, and in their place were found about twenty very active red-brown ciliates of different sizes, such as are shown in Fig. 109, C (× 250), after they had been killed by a weak osmic acid solution. The bodies of most of them were filled with the usual great corpuscles; some of them were distinctly pyriform in shape, and cilia were principally seen about the narrow anterior extremity. A portion of this specimen more highly magnified is to be seen in Fig. 109, D (× 250), which shows the great corpuscles within some of the Ciliates, and the pyriform shape of others.

Looking to the previously altered shape and appearance of the eggs, as shown in Fig. 109, B, and to what was found only twenty-four hours later in C, the most feasible explanation of this transformation, after all that I have previously seen, and have described in the last two sections is (*a*) that some of the eggs had been converted into great Ciliates such as are shown in Fig. 108, D; and that subsequently two processes went on, (*b*) the gradual devouring of the body substance of the parent organism, and (*c*) the multiplication by fission of the gorging Ciliates.

Happily a more recent observation has enabled me to prove the actuality of the occurrence of the first of these processes—namely, the conversion of the egg of the 'Sloth' into a single great Ciliate. The following details and illustrations will make this clear.

On May 9, a rather large Macrobiotus with five large eggs was found dead beneath a cover-glass, though it had been seen alive and with the same number of eggs only twenty-four hours previously. This creature is shown, looked at from above and with the eggs in focus, in Fig. 110, A (× 150). It will be seen that

instead of four eggs in linear series, the last but one is replaced by two eggs, side by side. There was no other 'Sloth' beneath this cover-glass, and fortunately it was lying alone, apart from other matter or organisms. After photographing the specimen, the microscope slip on which it was contained was replaced in the damp chamber. Three days later, at midnight, before putting away my work, I fortunately examined this specimen again, and found as I had seen on the previous day that the two anterior eggs had become much altered and in part disintegrated—their lining membrane, and part of their contents, had apparently disappeared, so that there was a light, single granular mass now in the situation which the two eggs had previously occupied. Now also the hinder egg was seen occupying more of the posterior extremity of the animal; and attentive examination showed that the whole mass was very slowly—almost imperceptibly—revolving within a limiting membrane, and occasionally a flicker of cilia could be seen. It was likewise clear that the whole mass was now largely composed of small corpuscles such as seem to be forming in the anterior egg shown in Fig. 109, B. As soon as these facts were clearly made out I photographed the specimen with a brief exposure, and the result is shown in Fig. 110 B, (× 150).

The slip was then replaced in the damp chamber with the addition of a little more distilled water to the edge of the cover-glass, and, bearing in mind the rapidity with which the changes had taken place in two other specimens of Macrobiotus, I determined to examine this specimen again after two hours. Accordingly, at 2 a.m. on May 13 I again put the specimen under the microscope and then found that there were two masses revolving, and rather more quickly, instead of one—the new Ciliate being situated anteriorly and partly concealing that which was first seen. This is only obscurely indicated by the photograph then taken, a reproduction of which is shown in C (× 150). I am disposed to think that the two had resulted from the fission of the one, and that it was only the posterior, and therefore the oldest, of the eggs which had become thus transformed.[1]

No examination was made again till after the expiration of seven hours, and what was found then is shown in Fig. 110, D (× 200). The two great Ciliates had by this time got into the anterior part of the body, and were revolving more rapidly. As I did not wish to kill them at this stage I took a photograph of the specimen at a low power (× 65) and with an exposure of twenty-five seconds, before returning the slip to the damp chamber.[2] It seems impossible to say from an examination of

[1] No such regular arrangement of the eggs has ever been seen by me as is represented by Doyère (*loc. cit.*) in Pl. xvi., fig. 1.

[2] This photograph has been since enlarged to its present size.

this photograph whether two, or only one of the Macrobiotus eggs (with subsequent fission) had become transformed, because the two Ciliates before they got away from the posterior part of the body had, by their movements, partly disintegrated the other eggs.

I examined the specimen again after another seven hours, and found still only two Ciliates, though they had grown, and were much more active. Evidence of their previous activity was also afforded by the disordered and fragmentary condition of the remains of the other eggs, and of the internal organs of the 'Sloth.' It was impossible to photograph the Ciliates again in their more active condition, and in my attempt to remove the Macrobiotus from underneath the cover-glass, where there was another specimen that I much wished to preserve, I unfortunately lost it. It is of course exceedingly difficult to turn up a cover-glass, causing everything beneath it to be displaced and intermixed, and find, again, amidst other matter, a minute object such as the one I was seeking. This, as I have said, was lost, though I succeeded in finding the other and rather larger object that I wished to preserve.

After all, however, the evidence that was wanted had been supplied—the egg of the Macrobiotus had been proved to be converted into a Ciliate. *The large stationary egg had become replaced by a slowly moving body of like size, which subsequently proved to be a great Ciliate.* The other two steps of the process by which in twenty-four hours such a specimen as is shown in Fig. 109, B, is enabled to yield the appearance represented in C is plain and easy—rapid fission of the Ciliate or Ciliates, together with their voracity, will account for all that C represents.

There is the further noteworthy fact that in various specimens of Macrobiotus that have been under examination in which there were no eggs, no Ciliates have ever appeared.

In the specimens shown in Fig. 110 the changes had been very slow in comparison with those shown in Fig. 109—seeing that after sixteen hours there were in the former specimen still only two Ciliates. This I am disposed to think was probably due to its having been examined three times, and photographed three times while the changes were in progress, instead of only once, as for the specimen whose changes are represented in Fig. 109.

Still the restrictive influence of light over these heterogenetic changes occurring in the eggs of Macrobiotus, is altogether slight as compared with the absolute stop which exposure to light puts to the heterogenetic changes occurring in the eggs of Hydatina when they are taken from the dark pot in which such changes have been initiated.

Whether the changes in the eggs of Macrobiotus which I

have just described will take place in the dark pot or not, as well as the question of the genus to which the Ciliate belongs which is thus originated, must for the present be left undetermined, as my supply of the lichen from which these particular 'Sloths' have been derived is now exhausted.

These changes in the eggs of the Macrobiotus are most interesting in another respect—seeing that they will take place beneath a cover-glass, though it is extremely difficult to obtain evidence that the closely-allied heterogenetic changes which I have described as occurring either with the eggs of Callidina or with those of Hydatina will occur under similar conditions.

LIST OF ILLUSTRATIONS.

SECOND PART.

A mere list of the Illustrations is here given, together with the Enlargements in Diameters of the several objects represented.

For the description of the figures the reader is referred to the text itself, at the pages indicated here, and on the plates after the numbers of the respective figures.[1]

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[1] The essential parts of different Photographs have been cut out and placed side by side in groups. The Plates in this Second Part are, therefore, really made up of 244 figures taken from separate Photomicrographs.

PLATE X.

PLATE XI.

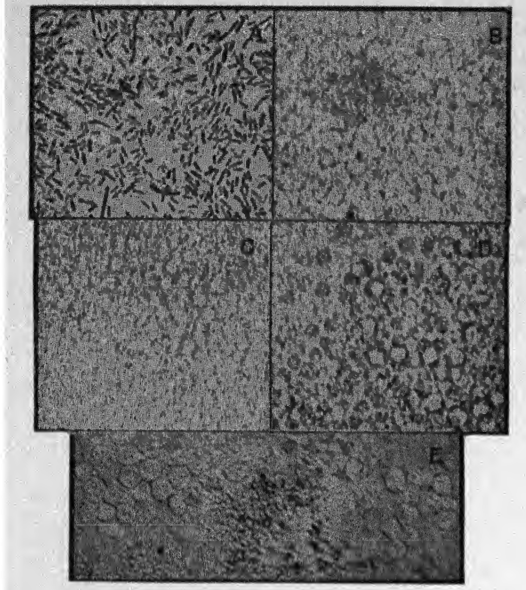


Fig. 53. pp. 67, 69.

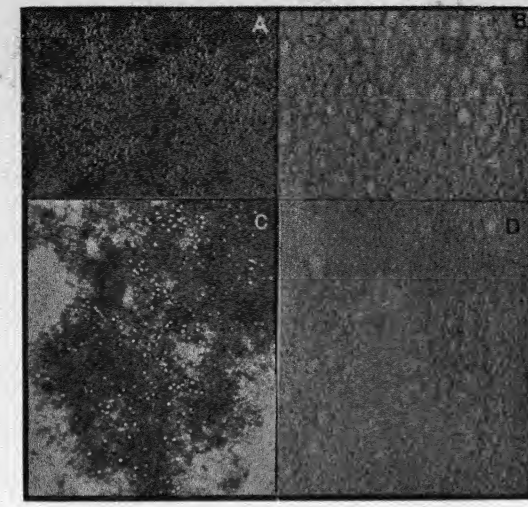


Fig. 55. p. 71.

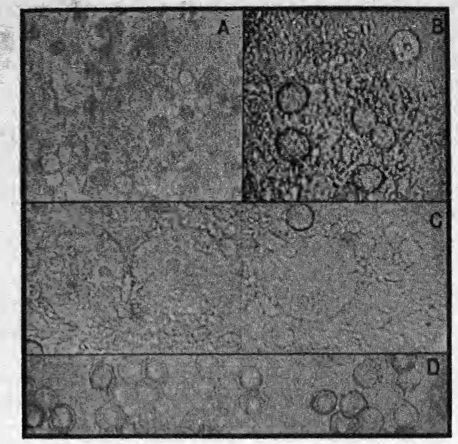


Fig. 56. p. 71.

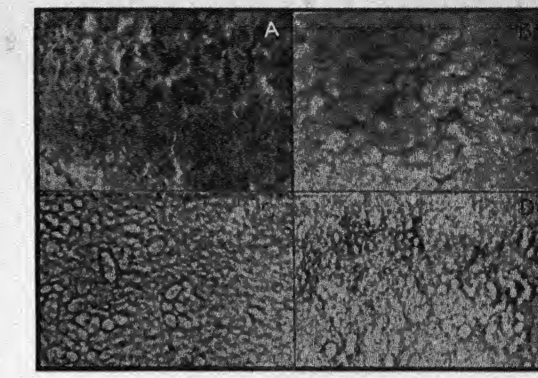


Fig. 64. p. 77.

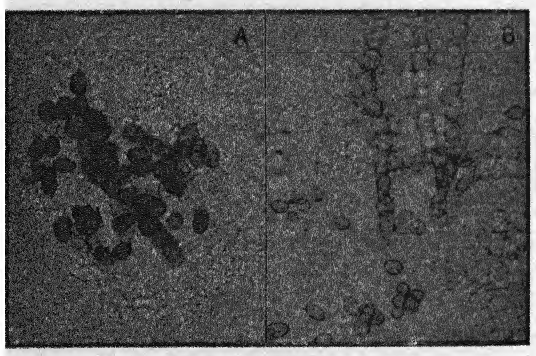


Fig. 59. p. 73.

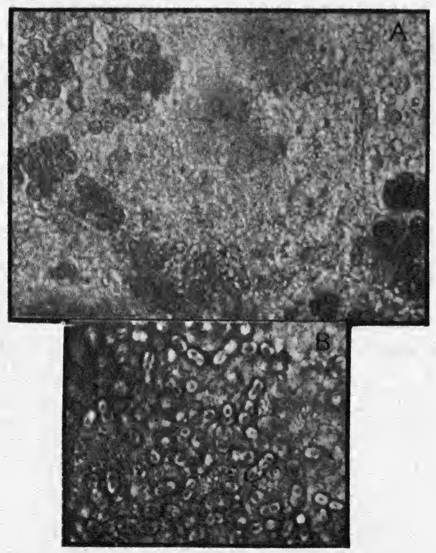


Fig. 61. p. 74.

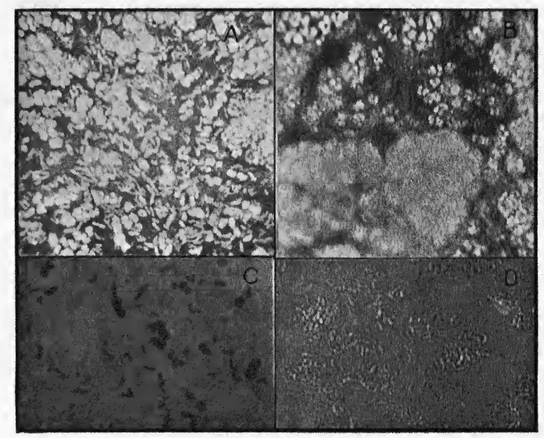


Fig. 62. p. 75.

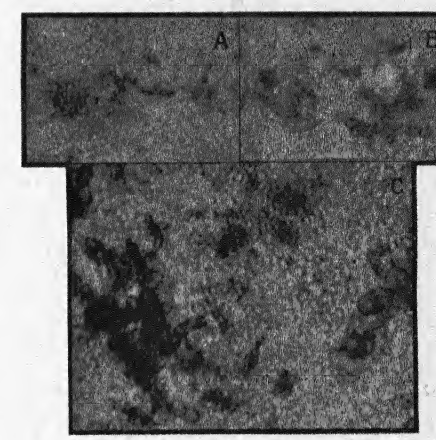


Fig. 60. p. 74.

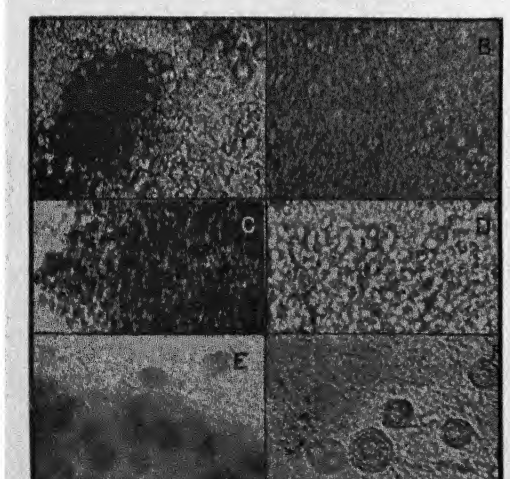
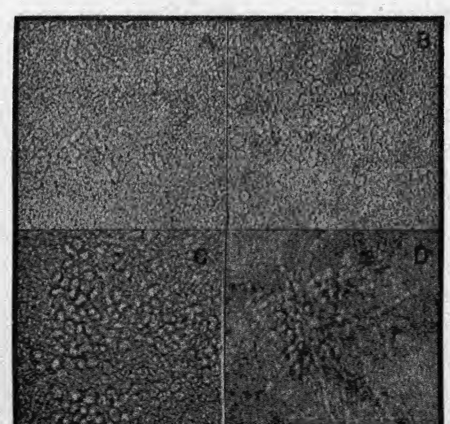


Fig. 57. p. 72.



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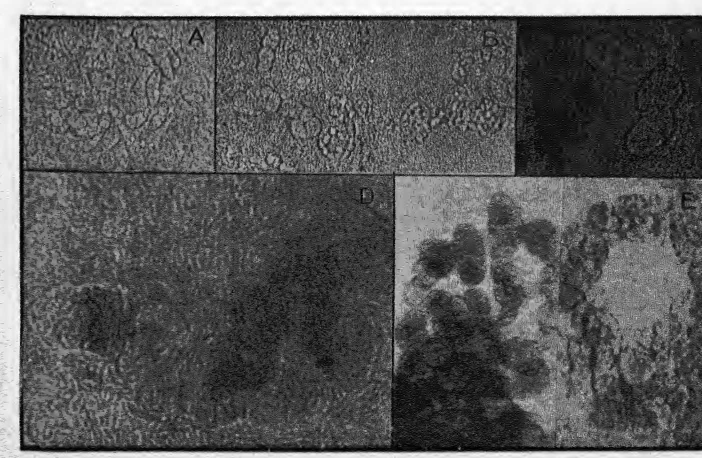

Fig. 63. p. 75.

Fig. 54. p. 70.

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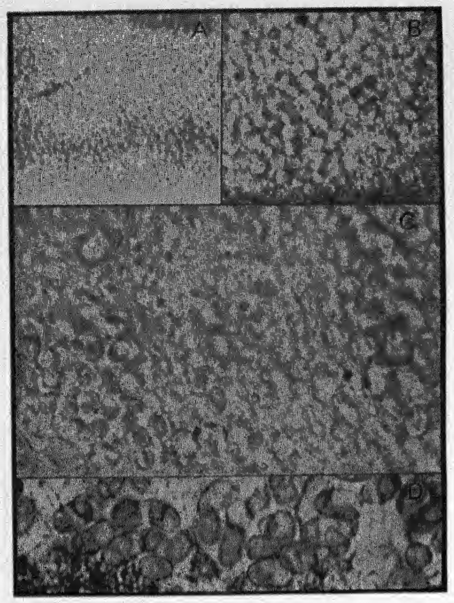


Fig. 65. p. 77.

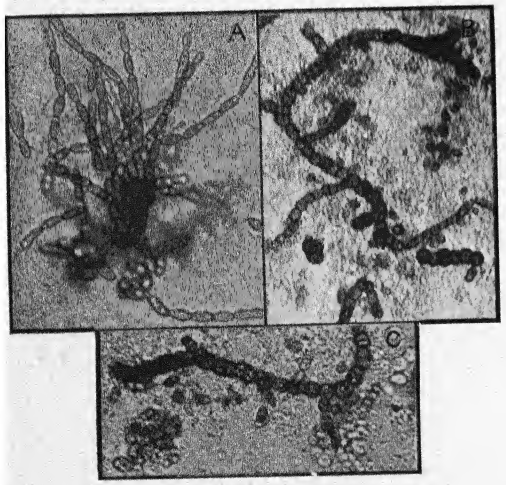


Fig. 66. p. 78.

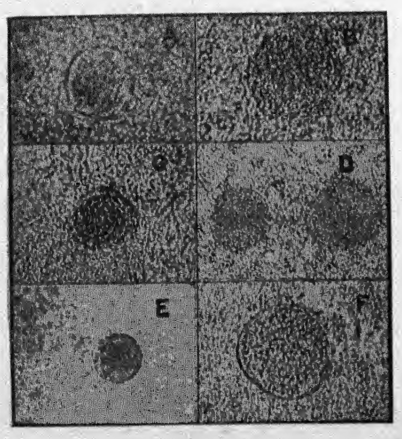


Fig. 73. p. 91.

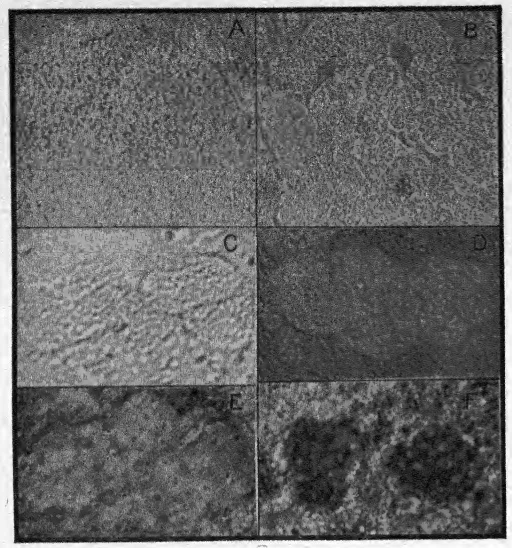


Fig. 67. p. 79.

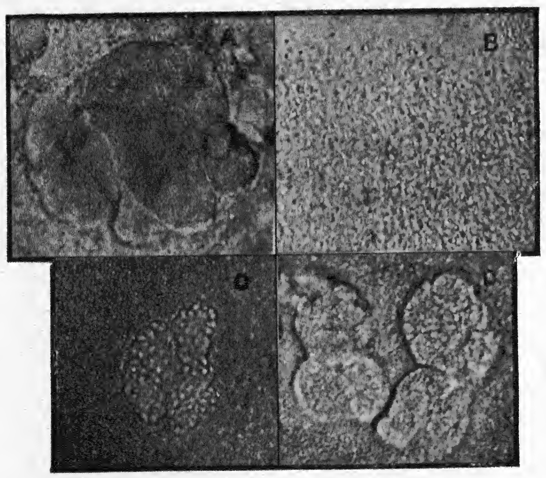


Fig 71. p. 84.

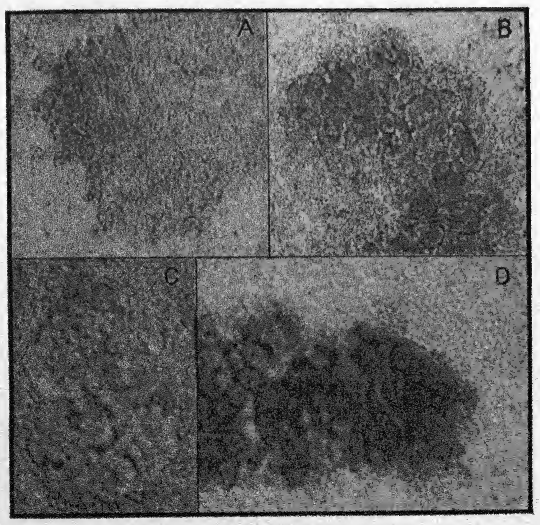


Fig. 68. p. 79.

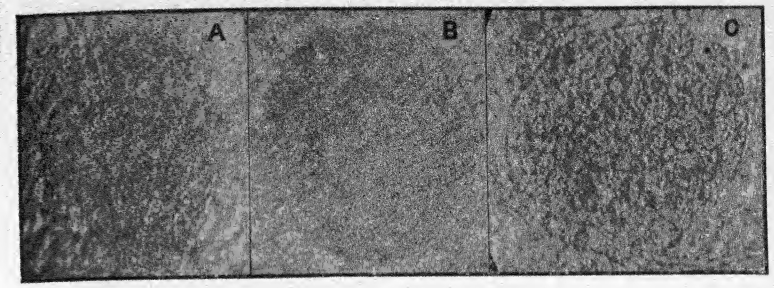


Fig. 75. p. 92.

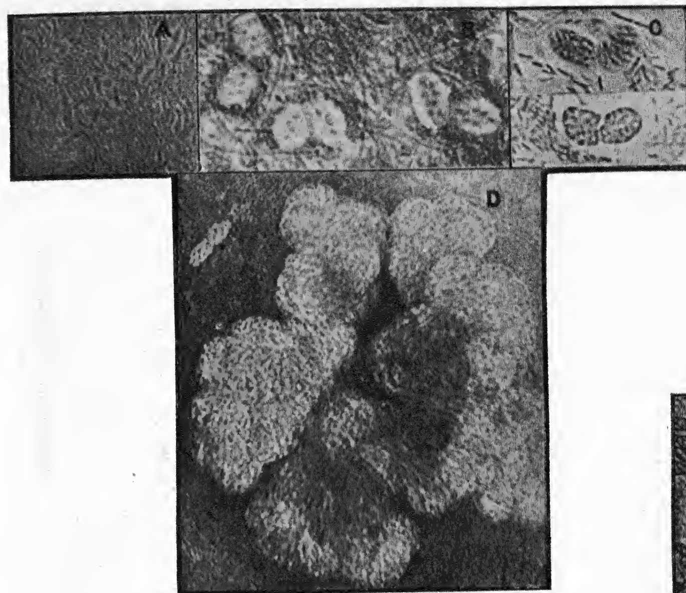


Fig. 70. p. 83.

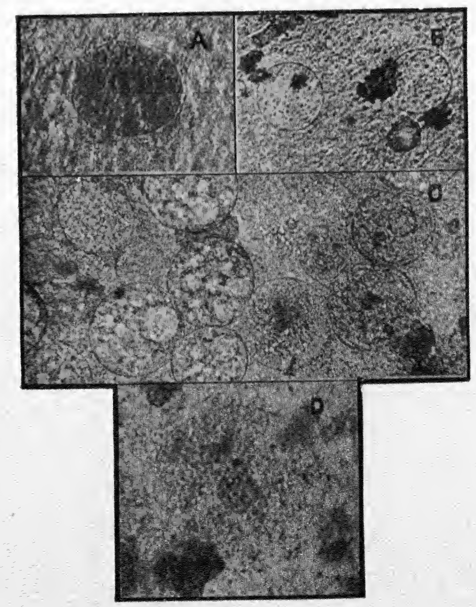
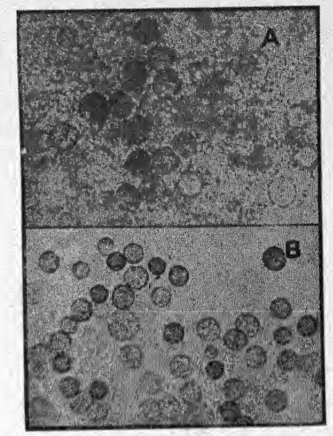


Fig. 74. p. 91.



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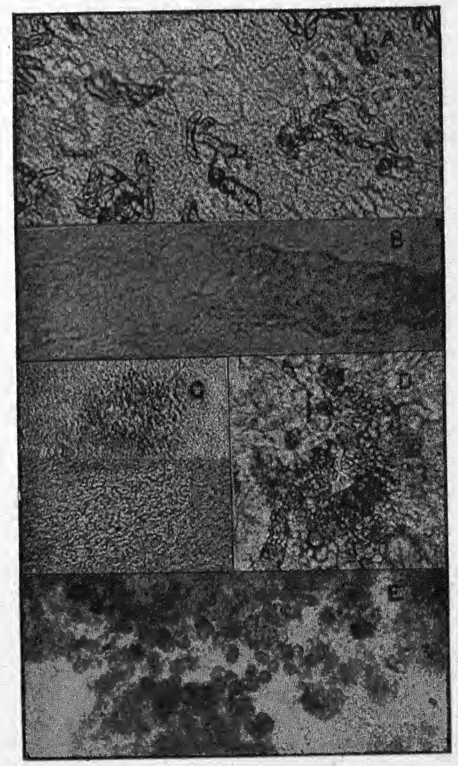

Fig. 69. p 80.

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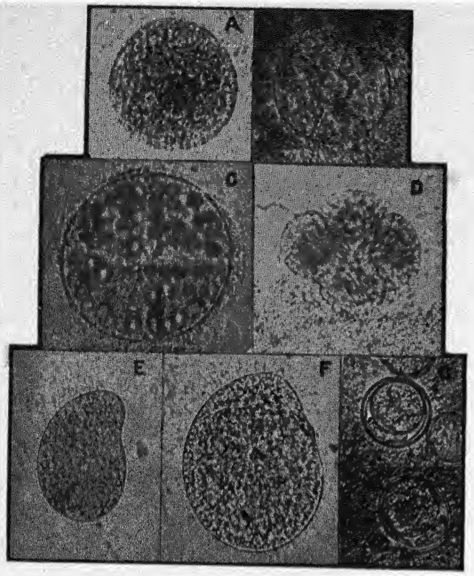

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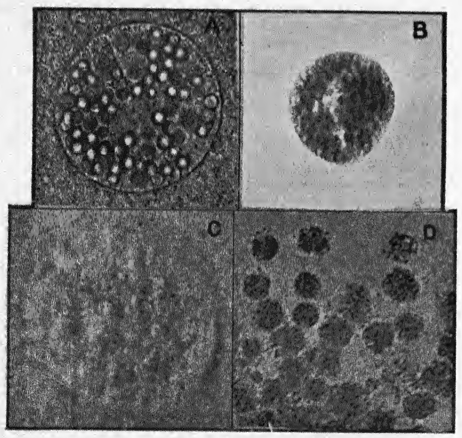

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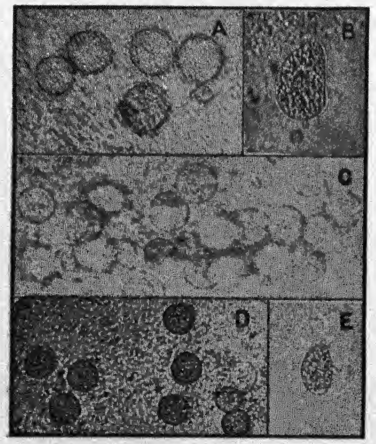
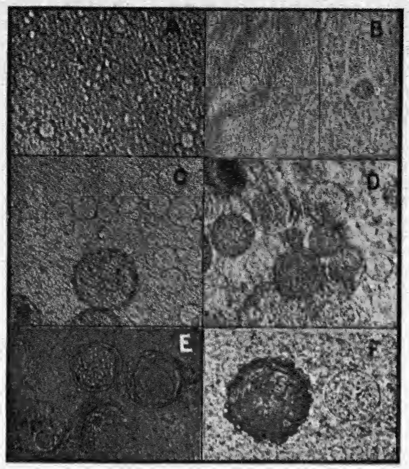


Fig. 79. p. 95.



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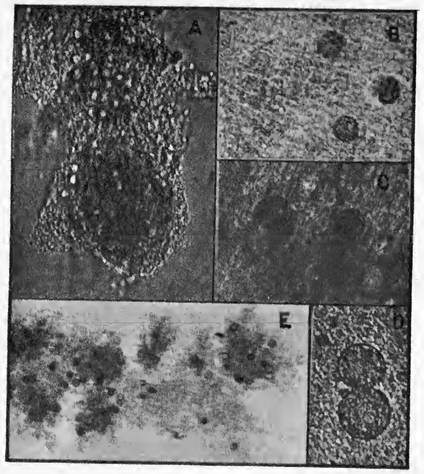

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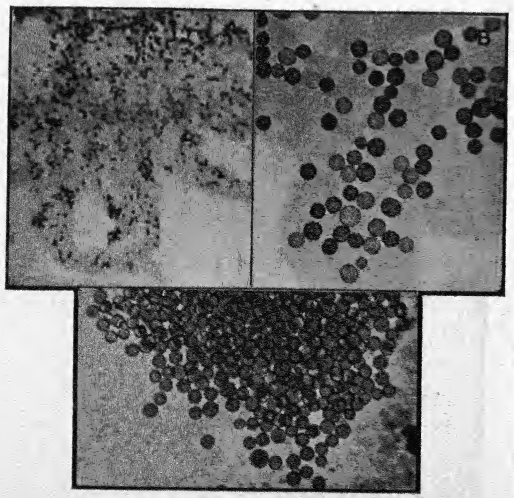

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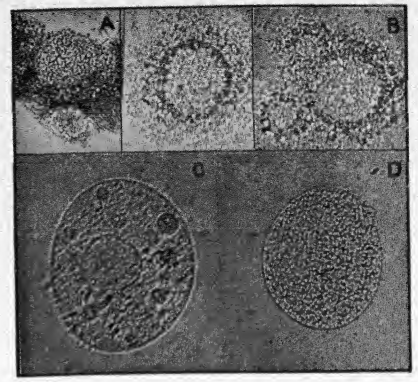


Fig. 84. p. 102.

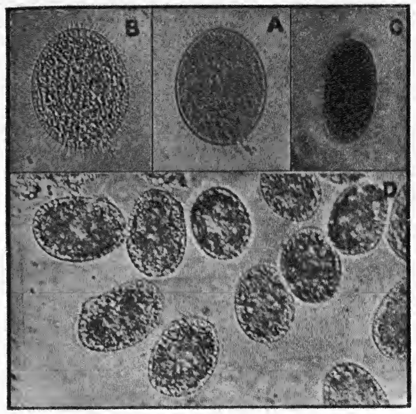
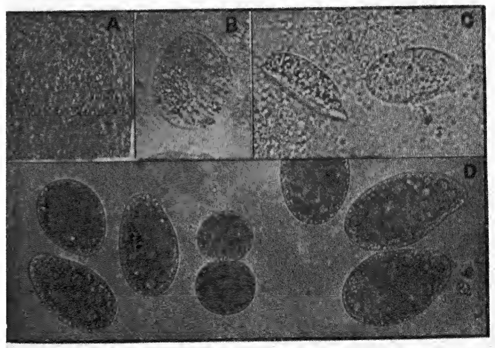


Fig. 85. p. 102



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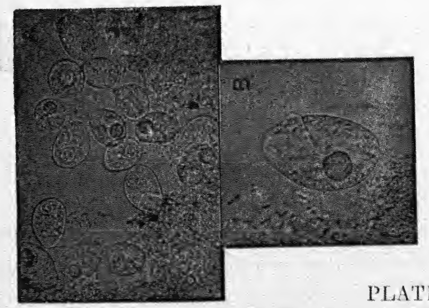

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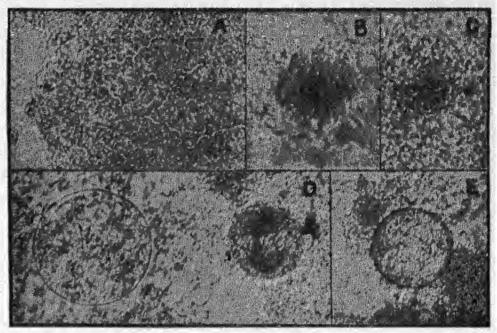

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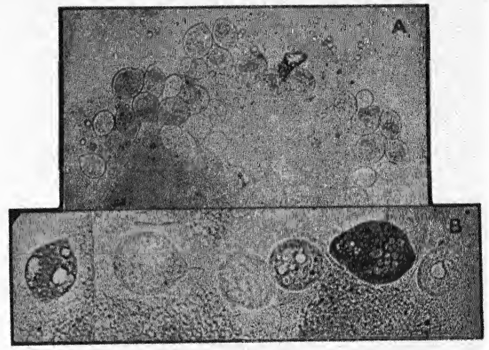

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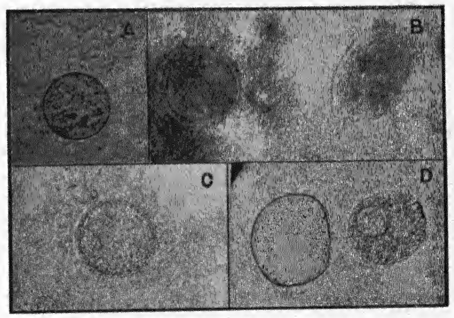


Fig. 88. p. 109.

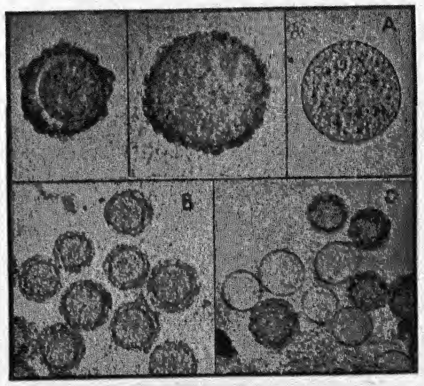


Fig. 81. p. 98.

PLATE VIII.

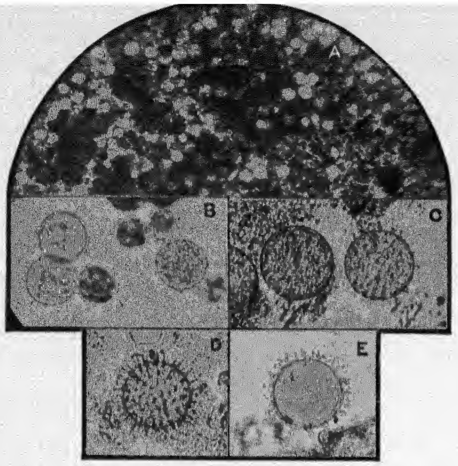


Fig. 90. p. 111.

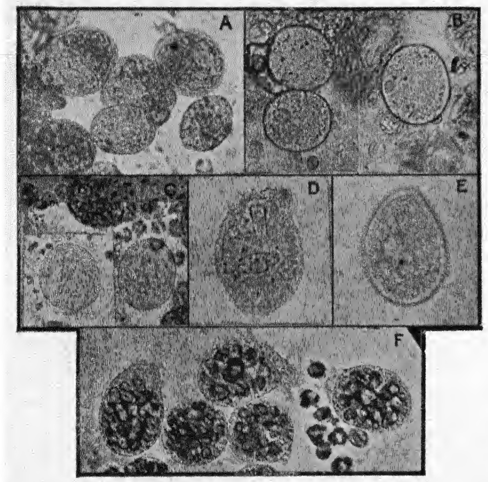


Fig. 91. p. 112.

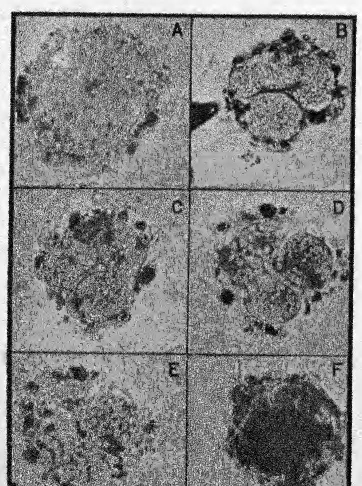


Fig. 94. p. 118.

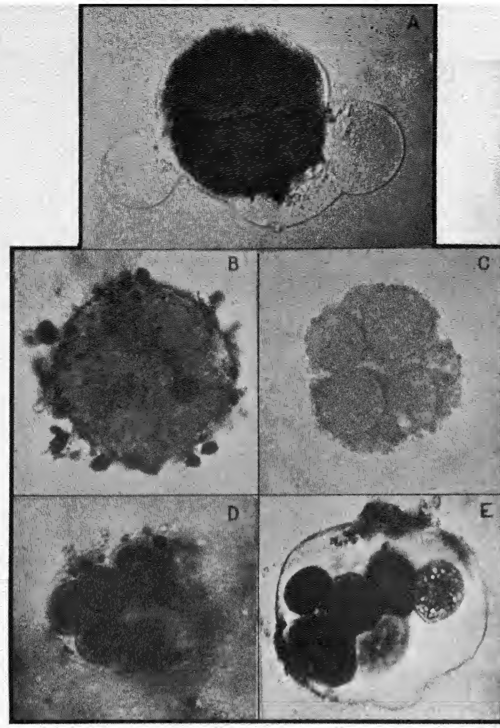


Fig. 92. p. 115.

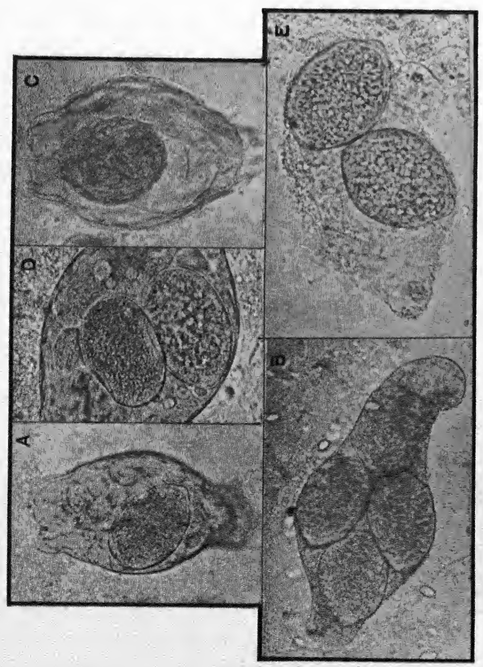


Fig. 95. p. 120.

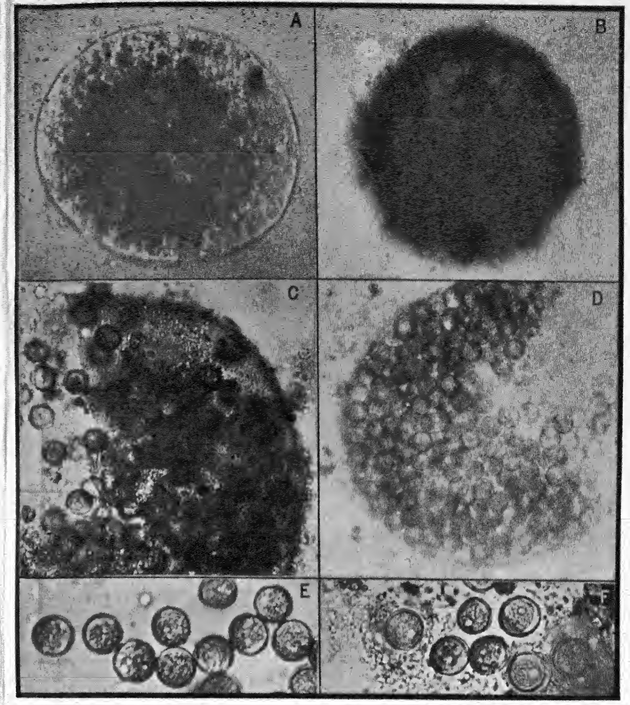
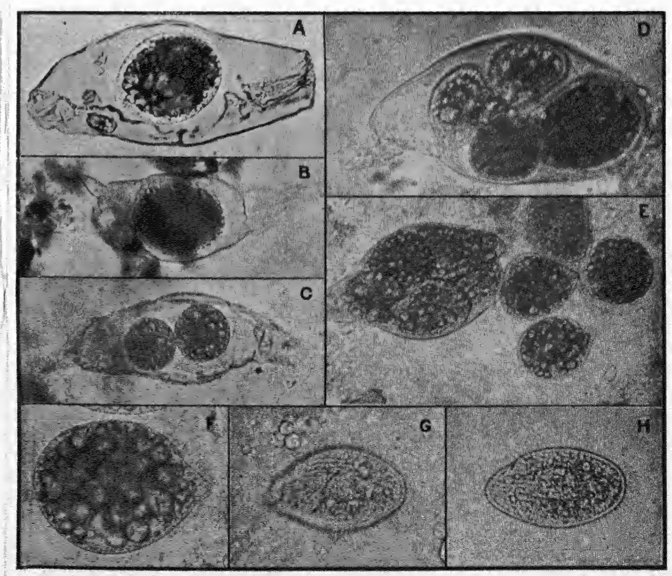


Fig. 93. p. 117.



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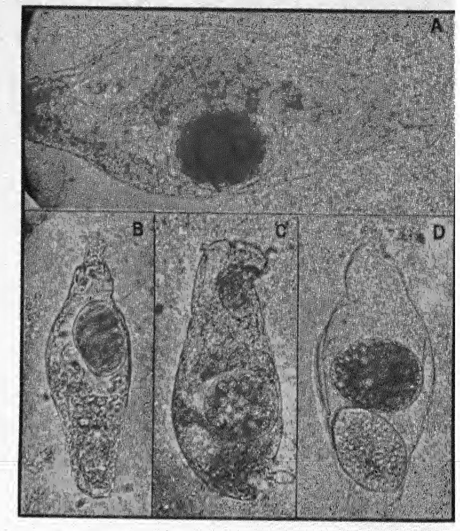

Fig. 97. p. 121.

Fig. 98. p. 122.

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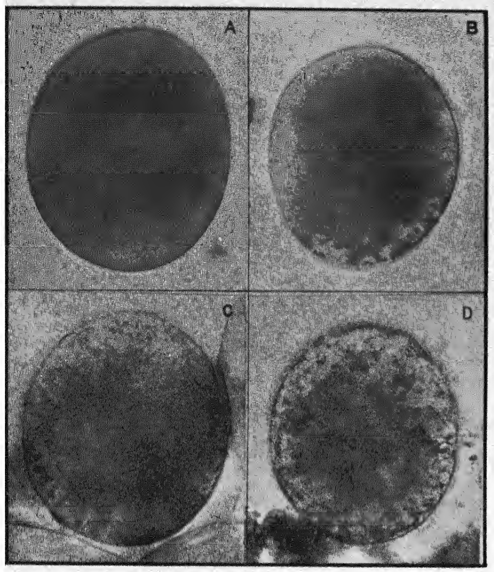
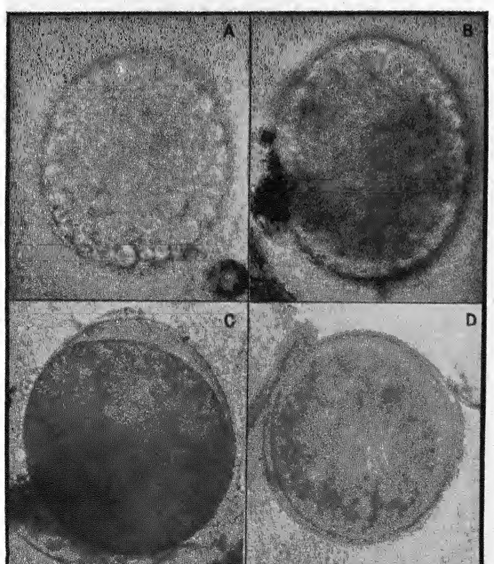


Fig. 99. p. 127.



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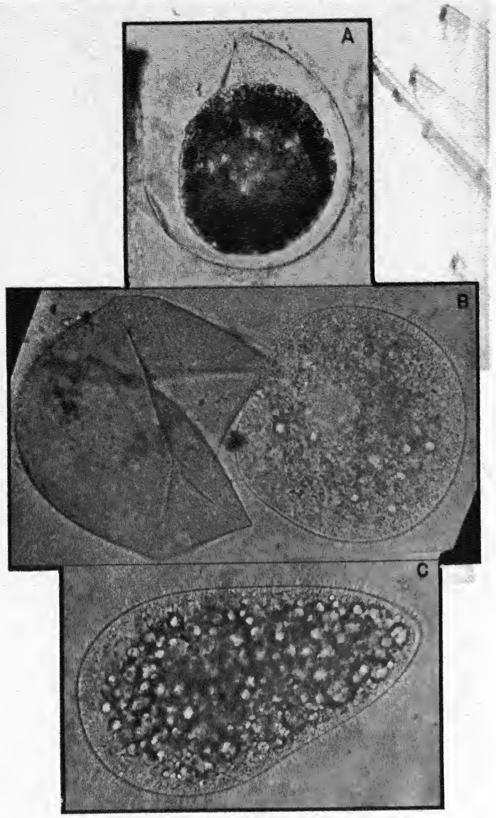

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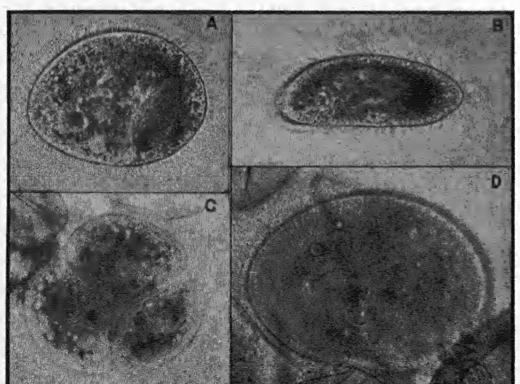
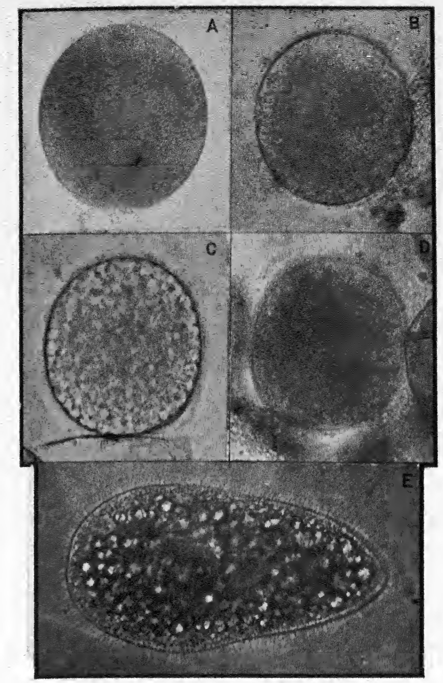


Fig. 102. p. 129.



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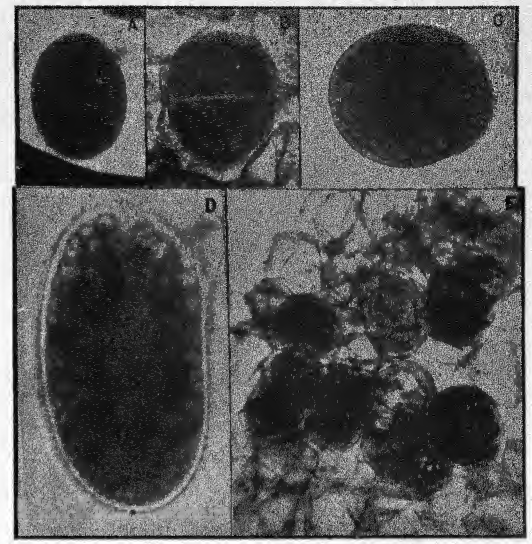

Fig. 104. p. 132.

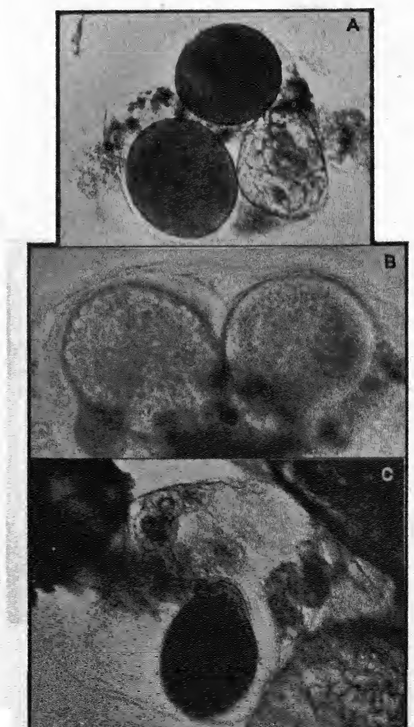

Fig. 105. p. 133.

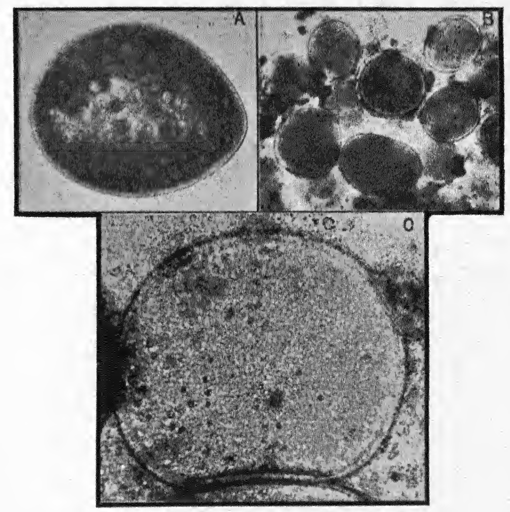

Fig. 106. p. 136.

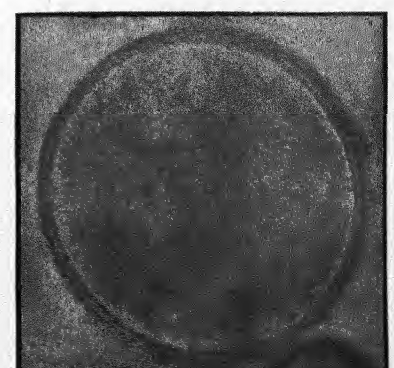

Fig. 107. p. 137.

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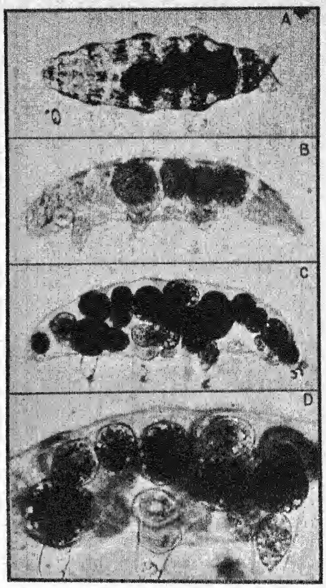

Fig. 109. p. 140.

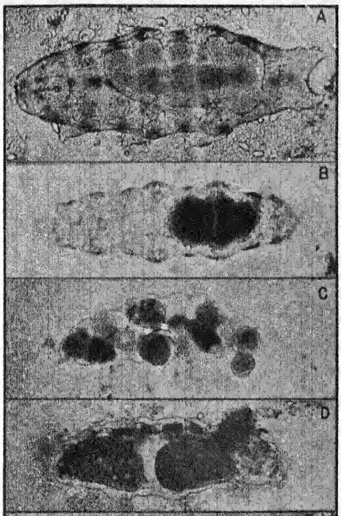

Fig. 108. p. 139.

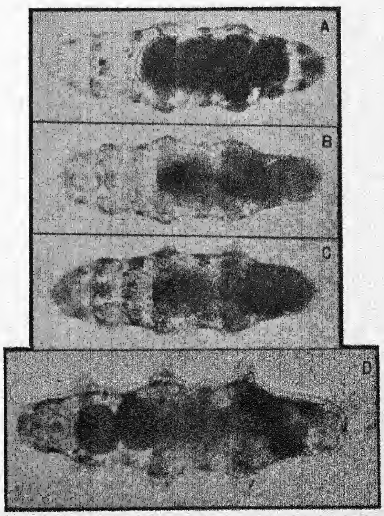

Fig. 110. p. 141.

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CONTENTS.

THIRD PART.

STUDIES IN HETEROGENESIS.

BY

H. CHARLTON BASTIAN, M.A., M.D., F.R.S.

THIRD PART.

XI. ON THE HETEROGENETIC ORIGIN OF DIATOMS FROM ALGOID CELLS.

In spite of the large amount of work which multitudes of naturalists have bestowed upon Diatoms much still remains to be ascertained concerning their life-history. The mode by which many of them effect their movements still remains a great puzzle. Then again representatives of a single species are often found in enormous numbers in this or that situation, varying greatly in size, though authorities on these organisms tell us that individual "diatoms do not grow."[1] Their enormous numbers are accounted for by most writers, such as Wm. Smith, Pritchard, and Wolle, by assuming that processes of fission take place with an entirely hypothetical rapidity. The first of these writers says:[2] "During the healthy life of the Diatom, the process of self-division is being continually repeated; the two half-new frustules *at once* proceed to divide again, each into two frustules, and thus the process continues. I have been unable to ascertain the time occupied in a single act of self-division; but supposing it to be completed in twenty-four hours, we should have, as the progeny of a single frustule, the amazing number of one thousand millions in a single month: a circumstance which will in some degree explain the sudden, or at least rapid, appearance of vast numbers of these organisms in localities where they were, but a short time previously, either unrecognised or only sparingly diffused." Pritchard writes in much the same strain, seeing that he says:[3] —"The rate of production of specimens of Diatomaceæ, even

[1] Wolle, "Diatomaceæ of North America," 1890, p. 11.
[2] "A Synopsis of the British Diatomaceæ," vol. i., 1858, p. xxv.
[3] "History of Infusoria," fourth edition, 1861, p. 60.

by this one process of simple self-division, is something really extraordinary. *So soon as a frustule is divided into two each of the latter at once proceeds with the act of self-division.*" Wolle expresses himself in very similar terms. But great exception may be taken to the words that I have italicised, as giving an entirely erroneous impression of the frequency with which self-division occurs. If it were true that self-division takes place so continuously as these authorities have represented, single specimens among the free forms should be the exception, while as a matter of fact they are overwhelmingly more frequent than such forms undergoing a process of self-division.

Other points on which much more information is needed concern the so-called "frondose forms," supposed to be produced by an "abundant secretion of mucus around the dividing frustules." These frondose forms are met with either "filamentous and more or less branched," "membranous and leaf-like," or forming "globose masses."[1] No adequate account exists in the work of either of the writers I have mentioned in regard to the production of these forms; and in the last and most elaborate work on Diatoms, Van Heurck's "Traité des Diatomées" (1899), not the least attempt is made to explain the aggregations of Diatoms in these frondose forms, though he quotes (p. 21) the statement of a very distinguished investigator, Dr. P. Miquel, to the effect that "the microscope does not permit one easily to distinguish this glue or this jelly in the midst of which, according to some observers, reproduction of Diatoms takes place." This remark refers especially to processes of rejuvenescence, or of conjugation and the formation of auxospores; but I can say from my own observation of three of the frondose forms of the filamentous type that no trace of mucus or glue-like matter has been recognisable with the many specimens that have come under my examination.

My observations, extending now over several years, enable me to account for the prodigious numbers of Diatoms, and their rapid appearance in new situations, not alone by reference to the rapidity of self-division; they force me to accept with considerable limitation the statement that individual "Diatoms do not grow"; and they furnish also another mode of accounting for the differences of size in numbers of Diatoms of the same species occurring often side by side. Finally, I hope to make some suggestions that may lead to further work, and a more satisfactory knowledge concerning the so-called "frondose" aggregates of Diatoms.

Some years since in examining a scum, composed in the main of Hæmatococcus corpuscles of different sizes, which had formed

[1] Pritchard, *loc. cit.*, p. 58, and Wm. Smith, *loc. cit.*, p. 7.

in a vessel containing some pond-water, these algoid corpuscles were seen in places closely intermixed with minute Diatoms of the usual brownish-yellow colour. There were patches of algoid corpuscles alone, patches of minute Naviculæ alone, and others in which these two bodies were intimately intermixed in various proportions. On closer examination one found that the algoid corpuscles and also the Naviculæ varied a good deal in size and, moreover, that the algoid corpuscles in places could be seen elongating, as in Pl. xii., fig. 111, A (× 250) and changing from a bright green to a brownish-yellow colour, and ultimately giving rise to such forms as are seen in B (× 250). The Diatoms here were mixed with yellowish algoid corpuscles in the lowest part of the patch.[1]

The same kind of thing was seen in an old Euglena pellicle where aggregations of minute algoid corpuscles of a bright grass green colour were found, and with them other similar but more ovoid corpuscles which had assumed a yellowish tint, and were intimately intermixed with small Naviculæ having a similar colour, such as are shown in C (× 375). And among the thousands of Diatoms seen in these situations one could only rarely observe any evidence of self-division. In the immense majority of cases it was single, not double, specimens that were recognisable.

Similar appearances have been seen on dead Vaucheria filaments, as shown in Pl. xii., fig. 112 (× 250). Patches of small algoid corpuscles occur in places, as in A; while contiguous thereto would be other patches of clustered Diatoms, as in B, or patches in which the Diatoms and the Algoid corpuscles were closely intermixed, and the latter changing in shape and colour and being apparently transformed into the former.

Certain observations, now to be recorded, tend to throw some light upon the possible mode of origin of some of the "frondose" aggregates of Diatoms. Many years since, when examining some specimens of Vaucheria, I first recognised what I then took to be dead filaments of this weed closely packed with Diatoms —all of the latter being of the same size and shape. From time to time since I have come across similar specimens, and have been much puzzled to understand the meaning of this strange association.

During the last two years I have learned more about it, and have ascertained that such specimens are most likely to be met with among the deeper layers of *V. terrestris* or *V. Dilwynii* when growing on a light soil, such as may often be found in the

[1] It is very unfortunate that the photographs make no distinction between the green colour of the algoid cells, and the brownish-yellow tint of the Diatoms, and therefore represent but poorly the transitions between them.

channels frequently made to conduct water away from a road to a ditch or water-course of some kind. At first, and indeed till very lately, I had much difficulty in finding, and still more in isolating, such specimens from a web of intermixed Vaucheria filaments under examination in the usual way beneath a cover glass. Raising the cover glass, to endeavour to get a specimen freer from surrounding matter, mostly meant the loss of the specimen altogether, or else the finding it again in a worse position than it was before. My early observations on this subject were, therefore, laborious and attended with great difficulty. Still, I gradually learned that these Diatoms were certainly not within old filaments of Vaucheria as I formerly imagined.

I found that intermixed among the roots, as it were, of these forms of Vaucheria, there was a small Microcoleus, the filaments or "trichomes" of which were of a greyish-green colour. They were contained in a sheath which was broad in the middle third but gradually tapered away towards each extremity. A half of one of these specimens is shown in Pl. xii., fig. 113, A (× 60) with particles of mud still adhering to its outer surface. All the specimens were of this nature, and were undivided, though they varied a good deal in length and also in breadth—owing to variations in the number of trichomes lying side by side in the middle third of the sheaths. A portion of one of these aggregates of medium size is shown in Pl. xii., fig. 114, A (× 375). A reference to Cooke's "British Fresh Water Algæ" made it clear that this was a species of Microcoleus, though different from either of the three recognised and figured by him as constituents of the genus,[1] and probably therefore one not hitherto described.

I gradually became convinced that it was within the sheaths of these specimens that the Diatoms in question were found. I at last succeeded in isolating several specimens similar to the one which is shown in Pl. xii., fig. 113, B (× 60). It will be seen that the general shape is that of the Microcoleus, and that the Diatoms were packed within a sheath of like kind, similarly broadest in its middle third, and tapering away towards each extremity. The nature of the Diatoms found within this sheath is shown in Fig. 114, B (× 375). The sheath itself in this specimen is covered with an unusual amount of granular matter, which, however, is absent from the smaller and less enlarged specimen shown in C (× 250).

A few specimens have been found in which the trichomes and the Diatoms existed within the same sheath, as in Pl. xii., fig. 114, D (× 250)—which is the only specimen that I succeeded in isolating sufficiently to enable me to photograph it, though I have seen several others in which the Diatoms and the trichomes were similarly associated.

[1] See *loc. cit.*, vol. ii., p. 254, Pls. 99 and 100.

The general shape of the Diatoms, and also the shape of the sheath, has a rather strong resemblance to *Colletonema vulgare* as figured by Wm. Smith (*loc. cit.*, Pl. lvi.). It appears that the valves in species of this genus have transverse markings, though Wm. Smith calls special attention (vol. ii., p. 70) to the fact that in *C. vulgare* such markings are "extremely faint and delicate." In their living state, and when full of protoplasm, I have never been able to make out any transverse striæ on the valves, though in some of the specimens I have detected very distinct, but faint, longitudinal markings. The Diatoms themselves have been tolerably uniform in size and always single; none have been seen in process of self-division. Some which I measured were found to be $\frac{1}{500}$ inch long by $\frac{1}{4000}$ inch broad. Generally they have the usual brownish-yellow colour; but occasionally the endochrome has been found to be of a pale green tint.

Up to the present I have been able to take the matter no further. I am unable to say whether the specimens I have just been describing really correspond to what has been known as *Colletonema vulgare* or not[1]; and I am further unable to pronounce any definite opinion as to the origin of the Diatoms, or the mode by which they obtain entry in such numbers into these Microcoleus sheaths. That the sheaths in which they are contained are Microcoleus sheaths seems evidenced by their general shape, size, and character, as well as by the fact that the Diatoms have several times been found within such sheaths in company with the Microcoleus trichomes. Still, as a rule, either Diatoms alone or Microcoleus trichomes alone are found within the sheaths. The two kinds being promiscuously mixed with one another and with the Vaucheria filaments.

If we are to suppose that the Diatoms get into the Microcoleus sheaths, we are met with two difficulties. How do they get in and pack themselves so tightly within these tapering sheaths? It may be supposed that a few get in and then multiply rapidly by self-division; but the specimens seen are always single specimens, and none have ever been seen by me within these sheaths in process of self-division. Secondly, supposing they are able to get in and multiply there, how do they get rid of the Microcoleus trichomes? This is a most difficult question to answer. Still it must not be forgotten that in the majority of cases nothing but Diatoms are found within the sheaths.[2]

[1] I have since this was written submitted the photographs to Dr. Van Heurck, and he kindly replies that, so far as the photographs enable him to judge, the Diatom is *Vanheurckia vulgaris*. He adds, "This form was wrongly placed by W. Smith in the genus Colletonema, from which it is absolutely different," and refers me for further explanation to his "*Traité des Diatomées*," pp. 238-39.

[2] It is also a matter of much importance that these Diatoms are only found with the Vaucheria within these particular sheaths or tubes. None are to be found free, except where a Microcoleus tube has been broken.

This latter difficulty would also have to be met if it were to be supposed that some other more actively motile bodies had obtained an entry within the sheaths, and had been there transformed into Diatoms. On such a supposition also we should have a right to expect that some of these unknown bodies, as well as the Diatoms to which they had given rise, would sometimes be found within the sheaths, either alone or in association with the Microcoleus trichomes. But, as a matter of fact, nothing but the Diatoms alone, the trichomes alone, or a mixture of the two, have ever been found within these doubly tapering sheaths.

If the trichomes could in some way have yielded the Diatoms and have been replaced thereby, these latter difficulties would have been got rid of. But of any such transformation having occurred I have no evidence whatever to offer.

Another possibility remains for consideration. Supposing I am wrong in imagining that these Diatoms are found in Microcoleus sheaths (in spite of the facts that the two are found together, that the sheaths agree in their shape and general characters, and that Diatoms and trichomes sometimes coexist within them), it might be contended that the Microcoleus is a thing altogether apart, and that the Diatoms constitute one of those aggregates of the "frondose" type in which the sheath or tube has been formed by the Diatoms during the processes of self-division which they are supposed to be so continually undergoing. These suppositions imply that the Microcoleus is one thing, and that the "frondose" aggregate of Diatoms (Colletonema) is something altogether different and unrelated thereto.

This opens up a question which I cannot help feeling is one of considerable importance in regard to the "frondose" Diatoms generally. In these, according to existing notions, the simple and branched tubes, the membranous expansions, and the globose aggregates, are all supposed to be produced by a mucoid secretion engendered by and disposed around the multiplying Diatoms. No detailed explanations whatever are given by the older writers, and the later ones, such as Wolle and Van Heurck, almost completely neglect this part of their subject. They scarcely refer to the question; they give no figures of the aggregates such as are to be found in the works of Wm. Smith and of Pritchard, and are principally concerned with the shape and markings of the individual Diatoms themselves. Yet, when one looks at the illustrations of these forms given by Wm. Smith in Pls. liv.-lix., and especially at those of the various forms of Schizonema, it seems obvious that there is much room for explanation as to the mode by which the simple process alleged

is capable of giving rise to such varied and complicated frondose aggregates.

I have myself never seen living specimens of Schizonema, and apart from the form previously described (presumably a species of Colletonema) the only other representative of the "frondose" Diatoms that I have met with have been the two species of Encyonema. They were found on the under surface of old Potamogeton leaves from a large pond near Northwood. Only portions of the tubes were seen among other matter scraped off from the under surface of these old leaves, so that I was able to make out nothing more concerning them except that the tubes had almost exactly the same kind of appearance as those in which the Colletonemas were found, and that in neither of them was there the slightest trace of anything like mucoid matter either within the tubes or around the Diatoms. The tubes or sheaths when old were covered with granules and smaller Diatoms as in Pl. xii., fig. 115, A. (× 250), which I take to be a specimen of *E. prostratum*. The Diatoms themselves in this specimen were dead and their endochrome was very dark and contracted. In younger specimens, however, the sheaths were perfectly smooth and diaphanous, as may be seen in the example of the smaller form shown in B (× 250)—which is probably *E. cæspitosum*. In these tubes the Diatoms were in single file and not crowded within the sheaths as is commonly the case with the Colletonemas I have seen[1]; and, as with these last, in the various specimens of Encyonema that I have met with none of the Diatoms have been found in the act of self-division.

What are we to say then in regard to the notion so commonly repeated by writers as to the frequency of the act of self-division among Diatoms? Where is the evidence that it goes on almost unceasingly? And, again, where is the evidence of this mucus produced by the dividing Diatoms, which is imagined to give rise to the tubes in the filamentous varieties of the "frondose" forms?. Certainly no trace of it has been found by me in the specimens of Encyonema and of Colletonema that have come under my examination. Some more detailed and definite work is evidently much needed concerning all these aggregates and their mode of production.

I now come to definite evidence proving that some Diatoms are frequently produced from transformed Algoid cells. What I have said hitherto only raises more or less strong presumptions that this is so. For, however cogent the kind of evidence first referred to may have been to the actual observer, it is not of a

[1] It is, however, by no means rare to find these Colletonemas also in smaller tubes and in single file therein, among the roots of Vaucheria.

kind whose mere narration would of itself suffice to bring conviction to others not believing in the possibility of any such transformations of species.

The mention of this evidence, however, for what it is worth, will be of use in showing that the more positively ascertained facts about to be recorded, may be found not to stand alone—when further patient observations have been made by persons whose minds are open to the possibility of the occurrence of such transformations.

In the instances to which I am now going to call the reader's attention, certain Algoid cells obtain an entry to, and multiply within, limited spaces or pockets in the substance of the thallus of different species of Duckweed, and there, after a time and under determining conditions very imperfectly known, some of such isolated groups of cells become transformed into Diatoms belonging for the most part to the genera Navicula or Nitzschia.

(*a*) On the Relations between certain Diatoms and the Fission Products of a parasitic Alga (*Chlorochytrium*).

Much interest was excited in 1872 owing to the discovery by F. Cohn[1] of an Alga existing as a parasite in the thallus of the ivy-leaf Duckweed (*Lemna trisulca*). This was followed in 1877 by the discovery of another parasitic Alga by Prof. Perceval Wright[2] infesting various marine Algæ. Since this time several other forms have been discovered, and rather an extensive literature has grown up concerning Chlorochytrium and allied genera. A key to some of this literature will be found in de Toni's "Sylloge Algarum," vol. i. (1889), p. 636, in which an attempt was made to classify the various species then known.

Among the new forms there is one *Ch. Knyanum*, found in *Lemna gibba* and in *L. minor*, which was examined and figured by G. Klebs[3] in 1881. This is evidently the Alga that I have of late met with very abundantly in both these species of Duckweed, and to which my present remarks will refer.

I find, during autumn and winter, among Duckweed from various localities, many dead and decolourised leaves, having a greyish-white and somewhat gelatinous appearance. Such leaves may be easily picked out, by spreading some of the Duckweed in a thin stratum of water over a white dish. It will then be found that the decolourised leaves are all devoid of rootlets, and possibly this loss of the rootlets may have been the main cause leading to the premature death and change in the appearance of the leaves.

[1] *Beiträge zur Biologie der Pflanzen*, Heft ii., p. 87.
[2] *Trans. Roy. Irish Acad.*, vol. xxv., p. 13.
[3] *Botan. Zeitung*, 1881, 248, t. iii., f. 11-15.

Examination with a hand lens, magnifying eight or ten diameters, will show in many of such leaves that the upper greyish-white surface is flecked with minute specks of an emerald-green colour, sometimes abundantly and sometimes sparsely: while examination of these or other leaves under the microscope will often show an abundance of the early stages of such bright green specks, so minute as to have been invisible with the mere hand lens.

It is best to pick out the smaller leaves for microscopical examination, and even then (especially with *L. gibba*) the examination can often only be satisfactorily carried out by placing one of the leaves in a drop of water on an excavated glass slip (taking care that its upper surface is uppermost) and gently compressing the leaf, if necessary, with the cover glass.

An examination of a very large number of these infected leaves has enabled me to ascertain the following facts.

The very active spores of the Chlorochytrium penetrate to some of the intercellular spaces of the leaf through the stomata. Single spores, or such bodies after a primary fission, may be seen just within the stomata (Pl. xii., fig. 116, A, × 250). Sometimes the entire spore, or the segments of the once or twice divided spore, will grow considerably before undergoing any further fission (as in B), though more commonly division goes on so as to produce eight or more cells which as they grow soon become tightly packed within the now dilated sub-stomatal space, as in C and D. Examination of the surface of the leaf over one of these patches will always reveal a stoma greatly dilated and almost circular in shape.

The mode of infection in *L. minor* and *L. gibba* is therefore altogether different from that described by Cohn as occurring in *L. trisulca*. In that species of Duckweed there is curiously enough an absence of stomata. The average shape and appearance of the patches of Chlorochytrium in *L. trisulca* is also rather different from that of the patches in the other two Duckweeds, and the latter patches also lack the distinct, and often thick, bounding membrane which occurs round the patches in *L. trisulca*.

In each of the forms the tendency is to an ultimate production of minute spherical or ovoidal zoospores, which after exhibiting a swarming movement may make their way out of the space where they have been developed. It often happens, however, in each of these forms of Chlorochytrium, that the zoospores may, either in whole or part, not succeed in escaping, but come to rest within their respective cells or spaces as shown in Fig. 117, B (× 375).

What I have further to say refers especially to *Ch. Knyanum*, and to this form as it occurs in *L. gibba*.

In some of the smaller patches composed only of two, or of four enlarged cells, it may occasionally be seen that segmentation of the contents of one of the cells only has occurred, while others have remained unaltered. This same kind of independence in the life of the cells occurs also in larger aggregates, some of the individual units of which may often be seen entire and undivided, while the contents of others are in different stages of fission, down to the final stage of spore formation.

It seems probable that sometimes the swarm-spores are formed by a simultaneous segmentation of the cell-contents into the brood of spores, but in other cases, as was clearly shown by G. Klebs[1] for *Ch. Lemnæ*, the cells undergo successive processes of fission till the swarm spores are produced. This latter kind of process I have found to occur very abundantly in *Ch. Knyanum*. Multitudes of partially empty spaces may be seen containing large or small specimens of these intermediate fission products, those within the same space being either all of one size (Pl. xii., fig. 117, C) or of very different sizes. Other spaces may be seen still full and distended with Chlorochytrium, the constituent cells of which exhibit very different degrees of segmentation, as in Fig. 117, A. Some have become resolved into the very minute zoospores (Fig. 117, B) while others have remained as fission products varying much in size. Some writers have spoken of some of the larger forms as being probably "resting spores."

It seems to me, however, that it can only with certainty be said that the Chlorochytrium cells undergo processes of division to a variable extent so as to yield fission products of very different sizes; and that, presumably under the influence of some unfavourable conditions in their environment, some of the products, at each of these stages, may undergo no further changes of a normal kind, and thus may never give rise to Chlorochytrium spores.

This brings me to one of the important points which this communication is destined to make known, namely, that in the latter stages of the life of *Ch. Knyanum* the fission products within the intercellular spaces of the leaf are often found to be more or less intermixed with Diatoms, varying not a little in size and in shape.

This association is met with sometimes in spaces none of the contents of which have escaped, and then the contrast is great between the beautiful emerald-green of the algoid cells and the brownish-yellow colour of the Diatoms mixed therewith. At other times partially empty spaces are seen containing the fission products of the Alga alone (Fig. 117, C), Diatoms alone (Fig. 117,

[1] *Loc. cit.*, taf. 3, f. 10, a, b, c.

D), or a mixture of the two kinds of units (Pl. xii., fig. 118, A, B, C, D).

More rarely spaces are found densely packed with brownish-yellow Diatoms only, in different stages of growth and development, except perhaps for the association of one or two minute algoid corpuscles (Fig. 119, A, B, C, D).

In regard to the Diatoms themselves these are sometimes very small and rudimentary, as in Fig. 118, A, and in the upper part of D, but at others they are much larger (as in Fig. 118, B, C, D), and these larger sizes are either fairly broad and ovoid like Naviculæ, or else narrow and elongated, like Nitzschiæ (Fig. 119, C, D).

In almost all cases however the Diatoms have the appearance of being immature; they have ill-developed siliceous envelopes, and are all quite full of brownish-yellow endochrome. There are also, at times, indications that growth and multiplication of these immature forms is, or has been, taking place, looking to the way in which they are occasionally ranged side by side in short rows in some of the half-empty spaces (as in Fig. 118, A, and in the upper part of D).

The sub-stomatal spaces which have been tenanted by the Chlorochytrium are characterised, as I have said, by a greatly distended and almost circular stoma, and often by having their walls stained of a more or less distinct rust colour. Indications of the latter change can be seen in Fig. 117, C, D.[1] It is a fact of much importance that Diatoms are never to be found in any of the sub-stomatal spaces except in those which either actually contain, or bear marks of having been previously tenanted by, Chlorochytrium.

Unfortunately I have found it very difficult to photograph some of the most remarkable specimens I have met with. This has been due to a combination of causes. It has been partly owing to the light having to pass through the whole thickness of the leaf, partly because of the staining of the walls of the spaces, partly because the photograph yields no discrimination in shade between the emerald-green colour of the Alga and the characteristic brownish-yellow of the Diatom; and at other times owing to the Diatoms being so closely packed within their little sub-epidermal pockets that their individual forms cannot be clearly shown—as in Pl. xii., fig. 119, in which two of the spaces (C and D) were closely packed with long and slender Diatoms like Nitzschiæ, while Fig. 118, C, D, contained broader and more ovoid organisms of the Navicula type.

It occasionally happens that the spores of the Chlorochytrium

[1] Of course these two characteristics, belonging to different planes, can never be seen together in the same photograph.

force their way from a closely packed space in which they have been produced, whence exit is not easy, in between various of the contiguous sub-epidermal cells, and occasionally in these situations I have also found Diatoms. Spores in such situations between the spherical cells are shown on the right side of Pl. xii., fig. 116, D.

There is another point of much interest to be mentioned.

Sometimes one of the epidermal cells, of zig-zag outline, will here and there be found filled by a light-green Alga, having the appearance of being a species of Chlorochytrium (Fig. 120, A, × 375). Other of these cells may be found in which such bodies seem about to undergo fission into several smaller cells (Fig. 120, B); and others still in which the original cell has divided into small green ovoid products (C), or into a number of more minute zoospores. In one case such zoospores were seen to have assumed a yellow colour and some of them seemed to be elongating, as was the case with some of the segments shown in Fig. 120, D. Many other of these isolated epidermal cells have been found containing either small ovoid Diatoms only (Fig. 120, F), or a mixture of such Diatoms with green fission products (as in Fig. 120, E), just as I have found the two kinds of bodies associated in the much larger sub-stomatal spaces.

The Diatoms in the epidermal cells are always small, commonly of about the same size, but not invariably so, and mostly having the appearance of being minute Naviculæ.

How the Chlorochytrium spores obtain an entry into these epidermal cells I am unable to state; but being actively motile it would clearly be much easier for them to get in than for the Diatoms to do so.

It seems most probable that it is the spores of *Ch. Knyanum* which infect these epidermal cells, and it seems possible that they may penetrate them from a sub-stomatal space, as I have often, though by no means invariably, found such infected epidermal cells just over, or by the side of, one of these spaces.

What interpretation is to be given concerning the Association of the Diatoms with the Chlorochytrium fission products?

Only two possibilities seem to present themselves:—

(*a*) The Diatoms have, like the Algæ, obtained entry to the sub-epidermal spaces through the stomata.

(*b*) The Diatoms have been produced *in situ* by a transformation of the fission products of the Alga.

The first of these possibilities it will be convenient to speak of as the Infection Hypothesis, and the second as the Transformation Hypothesis.

(*a*) *Infection Hypothesis.* The difficulty in accounting for the facts seems to me to be extreme in accordance with this

supposition, especially if we bear in mind what is authoritatively known concerning Diatoms. The important points are these:—

1. No *motile* spores are known, and previous to 1896 there was no certain knowledge concerning the existence of spores of any kind in Diatoms. The important discovery by Geo. Murray of undoubted spores or germs, originating by a process of rejuvenescence, in species belonging to three marine genera,[1] constitutes all that is certainly known at present on this subject.

2. It is commonly stated by writers that individual Diatoms do not increase in size[2]; increase in bulk of Diatoms being only brought about as a result of conjugation, which is admitted to be a comparatively rare process.

3. Previous to the above-mentioned discovery by Geo. Murray Diatoms were said to be formed only (*a*) by a process of conjugation, or (*b*) by fission, which latter is the common process, and one that is commonly said to involve a very slight diminution in size of the products.[3]

Such facts concerning Diatoms in general must be borne in mind in conjunction with these others more specially bearing upon the question now under consideration.

4. The sub-stomatal spaces which either are or have been tenanted by Chlorochytrium probably constitute much less than 10 per cent. of those existing on most leaves of the Duckweed, yet no Diatoms are ever to be seen in the other 90 per cent. of the sub-stomatal spaces.

5. The purposeless to and fro movements of some Diatoms when free in a fluid, and their absence of movement when lying on the surface of a leaf, seem quite incompatible with the notion of their selective penetration through certain special stomata only.

6. A point of still greater importance is the fact that Diatoms are never to be seen in the spaces in which the Chlorochytrium is in one of its early stages of development; they are to be found only in association with its later stages, where some of the final segmentations have been taking place, and often where the patches are so old that the walls of the spaces containing them are stained of a rust colour.

7. None of the Diatoms found either within the spaces or within their ramifications between surrounding cells have ever been seen to move.

8. Moreover where the Diatoms exist they are often intimately intermixed with the algoid cells; they are also to be seen in the ultimate ramification of spaces, even when these are still full;

[1] *Proceed. of Roy. Soc. of Edin.*, 1896-7, p. 207.

[2] Wolle, "Diatomaceæ of North America," 1890, p. 11.

[3] Smith's "British Diatomaceæ," vol. i., 1853, p. xxiv., and vol. ii., 1856, p. vii.; and Pritchard, fourth edition, 1861, pp. 58, 61-63.

and small specimens are likewise to be found between surrounding sub-epidermal cells contiguous to the invaded space. Such facts are incompatible with an entry of Diatoms from without if we bear in mind what has been said under the last two heads.

9. Again, where the Diatoms exist they not only vary much in size and shape in different spaces, but even within different regions of the same space.

Taken as a whole these various facts seem to me absolutely to negative the Infection Hypothesis as a means of accounting for the association of Diatoms with the fission products of Chlorochytrium in the sub-epidermal spaces.

(*b*) *Transformation Hypothesis.*—The facts which are so incompatible with the foregoing hypothesis will be found to offer no difficulties to, or to be capable of receiving a ready explanation in accordance with, the transformation hypothesis. This hypothesis is also strengthened by other facts not previously referred to.

1. The absence of the Diatoms from the 90 per cent. of the sub-stomatal spaces which are not infected by the Alga is explained.

2. The absence of movements on the part of the Diatoms in question affords no difficulty.

3. The absence of the Diatoms from the Chlorochytrium spaces during the early stages of the development of the Alga affords no difficulty and is explained.

4. The variation in the size of the Diatoms is explained, in the main, by the varying size of the fission products of the Alga. The two kinds of units very commonly co-exist, and where the algoid cells are small the Diatoms are small; where they are of medium or larger size the Diatoms are similarly of medium or larger size. Such variations in the size of the algoid cells are very common within the same infected space; and then, when Diatoms are present, they also are of various sizes.

5. Old, partially empty spaces are often to be seen containing the Chlorochytrium fission products small or large; others may be found containing Diatoms small or large; and others again partially empty, but containing a mixture of the algoid fission products with Diatoms of a corresponding size.

6. Other spaces still densely filled, will show with the algoid cells, Diatoms either packed in their midst, or occupying the boundaries of spaces, and often differing greatly in size in the two situations. They are likewise to be found occasionally in the narrow spaces between contiguous spherical cells, where, as I have said, algoid spores from the parent brood not unfrequently penetrate.

7. In the spaces where the algoid cells and the Diatoms are mixed, some of the cells may be seen to have assumed the brownish-yellow colour of the Diatoms, and some of such cells may also be seen more or less elongated, and apparently developing into Diatoms.

8. The majority of the Diatoms have an immature appearance. The siliceous envelope in the great majority of them seems to be either absent or very imperfectly developed; and unmistakeable evidence that multiplication of these immature Diatoms has taken place is frequently to be seen.[1]

There is no probability, and no one, I think, is likely to maintain, that Diatoms are normal phases in the life-history of this parasitic Alga; and as a careful consideration of the evidence, as a whole, appears utterly irreconcilable with the infection hypothesis, we seem unavoidably driven to the conclusion which is so congruous with all the facts, that the Diatoms in question are heterogenetic products actually produced by the transformation of the cells of the Alga—alike in the sub-stomatal spaces and in the epidermal cells.

I include the epidermal cells in this statement because almost all that has been said against the infection hypothesis and in favour of the transformation hypothesis, as accounting for the presence of the Diatoms in the sub-stomatal spaces, holds good also in regard to their presence in the epidermal cells. In one respect the argument is even stronger in its application to them, since there is much evidence to show that Diatoms are only found in those epidermal cells which are or have been tenanted by the Alga, and such infected cells never constitute more than the smallest fraction per cent. of those existing on the whole upper surface of a leaf.

A further point of extreme importance is to be found in the very great differences in the size and shape of the Diatoms, according as they originate from the small or the larger algoid

[1] Some of the differences in size, apart from those due to differences in the size of the algoid fission products from which the Diatoms originated, may be owing to actual increase of bulk in these immature organisms. Although this supposition is at variance with commonly received views, it is in accord with the observations of Geo. Murray, who says (*loc. cit.*, p. 216) that young Diatoms formed within a parent by a process of rejuvenescence, when liberated by "the separation of the parent valves at the girdle, may grow, divide and multiply before fully attaining the characteristic external sculpturing and adornment of the parent." Young Diatoms originating in fresh water may find silica in all pond water. The ammonia contained in rain water, like other alkalies, easily dissolves silica or aluminium silicate when in a finely pulverised state, and one or other of these compounds is to be found in all soils. (See Prof. A. M. Edwards, "On the Solubility of Silica," *The Chemical News*, January, 1896, p. 13.)

fission products. Yet these variations, for which no other contributary cause is apparent, are so great that botanists, unaware of the origin of the Diatoms and finding them in the Chlorochytrium spaces, would almost certainly regard some of them as belonging to different species of the same genus, and others even as representatives of distinct genera. This, however, is a subject which must be left for future investigation.

It was suggested to me by a distinguished botanist to whom I showed some of the specimens of Duckweed containing in their sub-stomatal spaces and epithelial cells mixtures, in various proportions, of Chlorochytrium segments and Diatoms, that their association might be explained by the Infection Hypothesis, backed by the assumption that *Chemotaxis* had been in operation —which, in this case, would mean that the physico-chemical processes associated with the growth and multiplication of the Algæ within the spaces were capable of giving rise to products exercising an attractive influence upon the Diatoms.

It was not pretended that there was any direct evidence in favour of this assumption; it was advanced as a possible explanation, and merely to stave off the conclusion, otherwise inevitable, that the Diatoms had been produced by the transformation of the cells of the Alga.

A careful and unbiased consideration of the following facts will, however, I think, make it plain that the evidence is overwhelmingly against Chemotaxis and the Infection Hypothesis:—

1. Chemotaxis can only be supposed to operate at short distances. But such Diatoms as are found within the spaces are never to be seen on the surface of the Duckweed.

2. The Diatoms that are commonly met with on the surface of the thallus (a comparatively large Navicula and a Cocconeis) are never found within the sub-stomatal spaces or the epithelial cells.

3. Chemotaxis implies a direct power of movement in response to an attractive influence. But none of the Diatoms on the surface of the leaf, within the spaces or within the epithelial cells, have ever been seen to move.

4. The Diatoms in the spaces are found intimately intermixed with the algoid cells, and generally in situations to which they could not be supposed to have the power of penetrating.

5. The Diatoms can often be seen to have replaced algoid cells, rather than to have pushed them aside.

6. Finally, in places, the Algoid cells can be seen elongating into the forms of the Diatoms, and at the same time changing from a bright green to a brownish-yellow colour.

Moreover, since making these observations on *L. gibba* and *L. minor*, I have ascertained that similar transformations of some

of the fission products of the Chlorochytrium which infests *L. trisulca* are also to be met with in that species of Duckweed. The Diatoms found in this species have been almost always very small and of the Navicula type—no Nitzschiæ having ever been seen in association with the segmentation products of this particular variety of Chlorochytrium, although the Duckweed bearing it has been taken from one of the same ponds from which I have obtained my supplies of *L. minor* and *L. gibba*. In Pl. xii., fig. 121 (× 375), some of the combinations that have been met with are shown. In A, four small spaces are represented. In the upper one Algoid segments and Diatoms are intermixed; in the one on the left young Diatoms were seen forming—the contents of this space being distinctly paler than those of the other two spaces in which the Diatoms were more fully formed and more closely packed together. In B two or three fused contiguous spaces are shown in which Algoid cells and Diatoms, together with various intermediate forms, were intimately intermixed; while C is the only space that I have yet found in *L. trisulca* containing Diatoms as large as are there represented. They were mixed with Chlorochytrium cells, as well as other minute Diatoms, though the latter are not recognisable in the photograph.

It is worthy of note that in *Lemna trisulca* there are no stomata. The active algoid spores penetrate, as F. Cohn showed, by boring between the epithelial cells into subjacent spaces, where they increase and multiply in practically closed cavities, and become also surrounded by a kind of capsule. Subsequently these active spores make their way out through very minute apertures which they themselves form, but in this species of Duckweed there are no widely dilated stomata through which in earlier or in later stages, should they attempt it, Diatoms would be free to enter.

Another point is also of much importance, and that is, the frequency with which Diatoms may be seen around the periphery of spaces still densely crowded with Chlorochytrium products which have not yet begun to emerge. These Diatoms, therefore, make their appearance within closed cavities, and often in regions far removed from the original point of entry of the active Algoid spore. No infection hypothesis, even backed by a further hypothesis of chemotaxis, is, I submit, capable of explaining the presence of these Diatoms. They are evidently formed where they are found by a transformation of the Algoid cells, and different stages of the process may often be clearly recognised—the spherical cells, as I have said, becoming elongated, and

changing from a bright green to a brownish-yellow colour as they take on the forms of the Diatoms.[1]

(*b*) On the Origin of Diatoms from mere Specks of extreme minuteness.

We have hitherto seen good reason to believe that processes of self-division are not so incessantly going on as authorities have represented,[1] and that the enormous numbers of Diatoms in some places may be in part otherwise accounted for—that is, by the occurrence of heterogenetic processes. There is, however, still another mode in which Diatoms appear upon the scene, and that by a process absolutely at variance with another dictum of most authorities who say, as Wolle does, that individual "Diatoms do not grow."[2] I maintain, however, that multitudes of them may unquestionably be seen growing on the surface of Nitella filaments and on various Algæ from specks so minute as to be scarcely visible with the microscope. As to the origin of these specks nothing can be positively said. As I have already stated, no process of minute spore formation is known to occur with Diatoms, and yet we shall see that they are constantly arising from minute germinal particles of some kind.

The origin of Diatoms in this way is, however, part of a larger question—one, that is, which concerns several other organisms—since Bacilli or Leptothrix, Toruloid elements, and Oscillatoriæ may be shown to have a similar mode of origin on the surface of such weeds from multitudinous minute germinal specks, none of which look as though they were free particles that had accidentally settled upon the surface of the weeds. They are always motionless, and have rather the appearance of growing in and from the surface layers of the Nitellæ or the Algæ.

We will refer to the simpler of these organisms first. It is extremely common to see old filaments of Nitella or Cladophora covered with a perfect forest of short or long Bacilli or Leptothrix threads. It is not easy to see what distinctions exist between these forms, seeing that Bacilli may be short or long. But these latter commonly lead a free existence, so we may perhaps reserve the term Leptothrix for the organisms growing from, and remaining attached to, surfaces of different kinds. In Pl. xii., fig. 122, c (× 375) such a growth from part of an empty Cladophora filament is shown. Such organisms are equally com-

[1] See also Sec. xiii., p. 183, concerning the origin of Diatoms from Algoid cells of another kind which are often found infesting the different species of Duckweed.

[2] "Diatomaceæ of North America," 1890, p. 11.

mon on *Nitella flexilis* or *N. translucens*. I have found too that if portions of these plants are kept for a time shut up in a dark pot we may often, on examination, see on their surface, instead of Leptothrix, a fringe of very minute Moulds, such as are shown under a low magnification in D (× 90). If we examine the surface of portions of filaments that are less old (after the contents of such filaments have been expressed in order to obtain a clearer view), we may see the beginnings of these Leptothrix threads or minute Moulds growing in the superficial layer of the integument of *Nitella flexilis* as minute whitish specks such as are represented in A (× 500), or still more enlarged from another specimen in B (× 800).

As I have said, they seem to be growing in the substance of, rather than merely on the surface of, the weed, and it is perfectly certain that the Leptothrix or the minute mycelial threads spring from such beginnings. Another point shown by the photographs, and one most worthy of note, is that these motionless germinal bodies are for the most part appearing and growing separately. What is shown by the photographs does not indicate a settling of germs on the surface and their increase there by processes of fission. They are not aggregated in groups as would most certainly have been the case had this been the mode by which they had made their appearance on the surface of the weeds. The weeds were, moreover, taken from water which was clear, and presented not the slighest appearance of turbidity. Yet if we look at the pretty uniform distribution of the germinal particles, and the comparative absence of aggregation here and there on the surface of these weeds (as seen under a magnification of 500 and 800 diameters) we might expect, if they were deposits from the water, that the water itself would have been turbid with such organisms; and, further, that from some cause, after they had been deposited, they had almost ceased to undergo processes of fission.[1] Then, again, spores are known to occur under certain conditions in Bacilli, but not in profusion in ordinary tap or pond water. Moreover, they are remarkably refractive bodies, larger and altogether unlike the germinal particles here shown on the surface of Nitella.

I will now refer to Oscillatoriæ and Diatoms, as they are often to be found growing together on different species of Nitella; though members of the genus Oscillaria are, in my experience, much less frequently found on Cladophora. On referring to Cooke's "British Fresh Water Algæ," it will be seen (vol. ii.,

[1] So far from the water being turbid, Fig. 122, A was taken from a cut Nitella cell after it had been for twenty-four hours in distilled water, which had remained perfectly clear.

p. 245) that he places the genus Oscillaria in the family *Lyngbyæ* The first genus of that family referred to is Spirulina, the description of the characters of which ends with the words, "propagation unknown." In regard to the next genus Oscillaria, nothing is said on this point; I therefore wrote last October to the veteran and distinguished algologist and asked him whether there was still a blank in our knowledge as to the existence of spores or germs in this genus, and his reply was, "I believe it is still true with regard to Oscillaria that propagation is unknown."

Reference may first be given to the mode of appearance of specimens of Oscillaria on these weeds. Some very definite observations have been made on old cells of *Nitella opaca*. A portion of such a cell is represented in Pl. xii., fig. 123, A (× 200), on the surface of which bluish or lead colour specimens of Oscillaria as well as minute brownish-yellow Diatoms were springing up in great numbers. The colours of the two which were so distinct in nature are unfortunately not differentiated in the photograph. These embryo organisms are accurately focussed along the middle of the filament. Some of those situated on each side, and out of focus, are represented as having a more colourless appearance. Some of the little spherical bodies growing from the surface of the weed had the colour of, and grew into, Oscillariæ, while others as they developed into Diatoms assumed characteristic colours and shapes. B (× 375) shows, with a higher magnification, on another portion of the same weed, specimens of budding Oscillaria more sparsely distributed but also more developed—the colour being most marked at the tip of the little Oscillaria buds. The large growth on the surface is a very common epiphyte on Nitellæ—belonging to the genus Entocladia.[1] Just above it, as well as below and to the right, some of these Oscillaria buds may be seen. The superficial substance of this Nitella filament was also thickly sown with the minute whitish germinal specks such as are better seen under a higher magnification in Fig. 122, A and B. Budding Oscillaria trichomes are also shown in profile, of the same colour, in D (× 375) growing from the surface of *Cladophora flavescens*. They spring up there also from minute lead-coloured germinal particles, such as are shown in C (× 500)—bodies which will be seen to be very similar to those found on Nitella. How these particles originate (as with those from which the Leptothrix threads develop) is at present altogether unknown.

The same kind of difficulty presents itself even in regard to one mode of origin of Ciliated Infusoria. In them, also, no minute germs have ever been discovered, and yet, as I have

[1] Described and figured by N. Wille in *Christiania Vedensk-Selsk. Forhands*, 1880, No. 4, Tab. 1.

shown (pp. 96-100), multitudes of these organisms may often be seen to take origin from minute specks of protoplasm, each of which gradually enlarges in size up to about $\frac{1}{500}$ inch in diameter or more, before it forms round itself a thick cyst, and undergoes further developmental changes, leading to the production of an active embryo that soon liberates itself as a free swimming Ciliate. Yet nothing is known as to the source of the germinal particles from which these Ciliates arise, seeing that no production of minute germs or spores is known to occur within the bodies of any of the Ciliata, and that they are believed to multiply by fission only. We are thus as ignorant on this subject as we are concerning the source of the multitudinous germinal particles from which Leptothrix filaments spring on the surface of Nitella and various forms of Algæ, either alone or in association with the sparsely occurring Oscillatoriæ.

I turn now to the question of the growth of Diatoms from similarly minute germinal particles. As I have previously said, on the dead filaments of *Nitella opaca*, portions of which are shown in Pl. xii., fig. 123, A and B, Diatoms were growing in abundance mixed with the specimens of Oscillaria. They originated from some of the dark specks shown in A, and gradually took on the shape and colour of the Diatom. The aid of a lens will also bring out the fact that a few of these embryo Diatoms are scattered over B (× 375). Over other parts of these filaments of Nitella the same Diatoms existed in extraordinary abundance, as is shown in Fig. 124, A (× 200). Multiplication by self-division had evidently greatly increased their numbers. Similar Diatoms, which seem to be forms of Pinnularia or Stauroneis, are shown in B (× 375) more enlarged and more sparsely scattered. Specimens of different sizes may be seen, from mere specks upwards, lying side by side and also undergoing processes of self-division. They are mixed in places with a species of Cocconeis, the representatives of which also vary notably in size, and to such an extent that we can only suppose some of them have been growing considerably.

I have made out much the same kind of thing with one ot the stipitate Diatoms, *Acnanthes exilis*, which I have found growing in the most extraordinary profusion on *Cladophora flavescens*, in association with another and larger species of Cocconeis. The Acnanthes seems to undergo self-division with extreme frequency, so that in some places it absolutely covers every portion of the filaments. Still, in places where the filaments have been cut and the endochrome discharged, I have been able to make out something as to the growth of this Diatom, and to ascertain that it increases, not only by self-division, but that it may also take origin in, and grow from, germinal particles of some kind situated on the surface of the weed. In Pl. xii.,

fig. 125, A (× 375) these Diatoms are shown, in profile and of full size, sparsely distributed over the border of a small stained cell of the Cladophora [1]; while in B (× 375) a group on another small filament (unstained) is shown of different sizes, in which the Diatoms are evidently growing and developing. In C and D (× 500) the same kind of growth and development is seen under a higher magnification. When the buds first show themselves they have a dusky appearance; as they grow they become ovoid, gradually assuming the form of the Diatom and showing two or three minute particles in their interior. At first they are sessile, and then the pedicle begins to develop. Among these bodies a number of circular markings are to be seen on the surface of the Alga having a nuclear-like body in the centre. What their nature is I am unable to say. I at first thought that these were the bodies that gave origin to the buds from which the Diatoms develop, but I have not been able to confirm this opinion and I have found such markings on old filaments on numerous occasions when they have been quite unassociated with the bud-like growths. Their nature must, therefore, remain uncertain.

What I have said and illustrated in regard to the appearance of Diatoms on Nitella and on Cladophora abundantly shows how erroneous is the popular notion that individual "Diatoms do not grow." It shows, too, that they often arise from germinal particles of some kind, although the source and mode of production of such particles is quite unknown to us. The multitudinous observations that have been made on Diatoms by a whole army of investigators have, up to the present, utterly failed to reveal the production of any such minute germs in these organisms. It must not be forgotten, however, that two investigators, Kitton [2] and S. Lockwood, [3] have made observations of an indirect kind which have led them to the conclusion that spores of Diatoms, so minute as to be capable of passing through very fine filter papers, must exist if any reasonable explanation is to be given of the results of experiments which they have made. Further investigations to throw light upon this obscure subject are evidently needed.

Meanwhile what I have made known as to the growth and development of Diatoms from minute germinal particles, and as to their frequent heterogenetic origin from algoid cells of different kinds, together with their multiplication by self-division, enables us to account for their rapid appearance in considerable numbers in many sites, without necessitating a belief in the extravagant statements that have constantly been made as to the rapidity and

[1] Some presenting the side and others the front view.

[2] *Journ. of Quek. Micros. Club*, vol. ii., 1885, pp. 178-9.

[3] *Journ. New York Mic. Soc.*, ii., 1886, p. 153.

frequency with which processes of self-division occur in such organisms.

XII. ON THE HETEROGENETIC ORIGIN OF DESMIDS FROM ALGOID CELLS.

Our knowledge of the life-history of Desmids is just as incomplete as that of Diatoms, and what has been hitherto known concerning their modes of increase resolves itself in the main into processes of self-division, that is, as Huxley termed it, mere "discontinuous growth."

The mode in which self-division occurs was clearly described long ago by Ralfs,[1] and his description is generally recognised as correct. The halves of the dividing organism are gradually separated and the portions which are to form new halves "increase in size, acquire colour, and gradually put on the appearance of the old portions," and, "as they increase the original segments are pushed further asunder, and at length are disconnected, each taking with it a new segment to supply the place of that from which it has separated." From the existence of this mode of division it follows that where the separations are incomplete, as in one of the filamentous Desmids, "the two oldest segments are found at its opposite extremities."

Ralfs supposed that the reproduction proper of Desmids occurred in two modes, "one by the escape of the granular contents of the mature frond, and the other by the formation of sporangia, the result of the coupling of the cells." On the next page, however (p. 10), he says, "with the subsequent history of the granules I am altogether unacquainted, but I conclude it is similar to what has been traced in other Algæ."

This supposition of Ralfs that the well known "dancing granules" of Desmids are reproductive particles has been absolutely rejected by subsequent authorities. Cooke, for instance, strongly dissents. He says[2] these "dancing granules" of Closterium "remain unchanged by burning or by treatment with concentrated acids and cold alkali. At all events they answer to inorganic bodies." Other small granules in the cell fluids of a Cosmarium, he says, "have a different composition, for they will dissolve readily in reagents, and are destroyed by burning, but are insoluble in alcohol."

Cooke knows nothing as to the production of minute germs or "zoospores" in Desmids. He says (*loc. cit.*, p. x.), "Although it has been stated in vague general terms that zoospores are produced in the Desmids, the only foundation for the assertion

[1] "The British Desmidiæ," 1848, p. 4.
[2] "British Desmids," 1887, p. vi.

appears to have been the presence of these bodies in genera now excluded from the Desmidiaceæ." He can only refer to an unconfirmed observation of W. Archer made twenty-seven years previously on a species of Docidium in which that observer believed he had found zoospores. But, though believing that the bodies in question were zoospores, Archer frankly admitted that he was "quite ignorant of their after development." It may still be said, therefore, that no germs or zoospores of the Desmidiaceæ are known, and that reproduction proper only takes place as a result of coupling, with the formation of a sporangium or zygospore.

These sporangia are by no means common, and they seem to be products whose morphological characters are extremely variable, not only in different genera but even in different stages of development of the same body. Ralfs wrote as follows concerning them :—"In many genera the sporangia remain smooth and unaltered; in others they become granulated, tuberculated, or spinous—the spines being either simple or forked at the apex. In fact a sporangium may pass successively through all these stages, and hence may so change its appearance that its different states are liable to be taken for sporangia belonging to different species." The subsequent history of the sporangia or zygospores is, indeed, very obscure. Cooke says (*loc. cit.*, p. ix.) "the development of new individuals from the zygospore seems to be rare"; while Wolle[1] in reference to the same subject, says, "germination is, if not of rare occurrence, very rarely observed." The germination has, in fact, been scarcely ever seen. Ralfs gives one figure (Pl. xxvii.) showing a number of small Closteria within a cyst, but he knew nothing definite as to its origin, and doubted whether it was in reality a germinating sporangium. Both Cooke and Wolle give very many illustrations of sporangia belonging to different genera and species of Desmids in their very numerous plates, but they do not give, and therefore we may assume were not able to give, from their own observation, a single illustration of the development of young Desmids from the zygospores.[2]

The only real description of the development of Desmids from the contents of a sporangium after such a fashion that I have found is that given by Hofmeister,[3] the stages of which were partly watched in *Cosmarium tetraopthalmum*, but more completely in *C. undulatum*. In regard to this latter organism

[1] "Desmids of the United States," 1892, p. 18.

[2] Wolle has one figure (Pl. xxvii., fig. 33), like that of Ralfs' of a few small Closteria within a cyst with this description: "A cyst containing a number of small Closteriums; *supposed to be the development of a zygospore.*"

[3] A translation of whose paper is to be found in the *Annals of Natural History*, 1858, vol. i.

he says: "In this again I observed the contraction of the green contents of the cell into a globule occupying the central part; the division of this ball into two, four, eight and sixteen spherical masses; finally the transition of these daughter cells of the last generation from the form of circular lenticular bodies into two-lobed ones like the mother plant. Here the young Cosmaria, whose diameter amounted to scarcely $\frac{1}{5}$th or $\frac{1}{4}$th of that of the mother plant, were set free by the very gradual solution of the membrane of the spore."

It seems quite possible, therefore, that the cysts figured by Ralfs and Wolle containing a few small Closteria, to which reference has been made, may have been the delicate inner envelopes of zygospores from which one or more thicker and coarser layers had in course of time been thrown off, this being a process which some observers have described.

The only other description of the formation and germination of a zygospore that I have seen is that by de Barry[1] of the process as it occurs in *Cosmarium Botrytis*. But here the process is different, as the zygospore produces only a pair of rather large Desmids, instead of many small ones.

It comes to this, therefore, that, apart from the observations of Hofmeister and de Barry, we know next to nothing of the actual reproduction of such common organisms as Desmids, and the derivation of young organisms from the zygospores. That the process must be a comparatively rare one is further shown by the fact of the rarity of minute Desmids of any species—that is, of specimens very much below the ordinary size commonly attained by its representatives. Of course if the formation of Desmids from minute germs had been common in the sporangia, then, as with so many other organisms, we ought constantly to be finding forms between such minute germs and the fully grown parents. But this is notably not the case.

It is surprising, too, even when comparatively large numbers of any kind of Desmid come under observation, how uncommon it is for specimens to be seen in the process of self-division. My experience in this respect does not seem to be very different from that of Cooke; all that he says on this subject being as follows: "The increase of cells in the Desmids by ordinary cell-division is by no means uncommon"—a guarded statement contrasting notably with what most authorities have said concerning the extreme frequency of the process with Diatoms.

Apart from the comparative absence of minute forms of any particular Desmid, and the way in which one constantly finds them approximately equal and of adult size, I have been much struck with the fact that many specimens when of this full size

[1] Quoted and figured in Sachs' "Textbook of Botany," 1875, p. 221.

are frequently to be met with in very different stages of development—that is, of internal differentiation. I have several times seen this with specimens of Closteria. On one occasion I found thousands of them forming a green film on the surface of damp mud. Some of this was gathered and on examination there was nothing but a dense array of these Desmids mixed with comparatively few filaments of Vaucheria. In the majority of the Desmids the endochrome had almost exactly the same colour and granular appearance as that in the Vaucheria filaments. There was no differentiation of the contents into bands with a row of amylum bodies along the centre, such as were seen in a much smaller number of developed forms associated with the others. In these more differentiated specimens the endochrome seemed to become gradually rather paler and more homogeneous, while the amylum bodies began to develop, showing themselves first at each extremity of the organism.

Although none of the Closteria were seen actually in process of division, yet a good number of the undifferentiated specimens were composed of unequal halves, indicating that division had recently occurred. Still in these cases the larger was just as undifferentiated as the smaller segment.

Much the same kind of variation in regard to internal differentiation was found on another occasion with some Closteria associated with *Cladophora flavescens*, though with none of these organisms were specimens seen indicating that division had recently occurred. Some indication of the differences referred to is shown in Pl. xiii., fig. 126 (× 125), even under a low magnification. Thus, A represents an undifferentiated specimen in which the endochrome was abundant and granular, the median space was very small, and in which there was no appearance of amylum bodies; in B a later stage apparently is shown, the endochrome was less densely granular and a row of amylum bodies was beginning to show itself; while in C the Closterium was fully developed, the endochrome having become more band-like and homogeneous and the row of amylum bodies much more distinct. Yet these three organisms are all of the same size.

On another occasion, during the course of some days, I came across more than a hundred specimens of some species of Cosmarium in a shallow vessel containing a quantity of *Ulothrix variabilis*. All the specimens were approximately of the same size; none were in process of self-division; and only one was seen in which the segments were unequal, indicating that such a process had lately occurred. Yet specimens were found differing very notably in their degree of internal differentiation, as Fig. 127 (× 250) shows. Some were like A, densely packed with endochrome of a blackish-green colour; others like B not quite so densely packed and the contents having a somewhat mame-

lonated appearance; others as in C showed the dark green endochrome more divided and, in the middle of each lobe and elsewhere, resolved into "swarming granules"; while in D this change had become more complete, the endochrome being arranged in each segment into three principal masses, large portions of which had been resolved into "swarming granules." These were situated in the regions which in the figure are of a lighter colour, and were principally seen in irregular cavities formed in the central mass of endochrome, though they were distributed also more diffusely at the sides.[1] In other specimens the endochrome was of a paler green, no swarming granules were anywhere to be seen, the contents being more homogeneous and more scanty, so that the organism had rather a starved appearance.

On another occasion I found some very minute Cosmaria growing on the filaments of *Cladophora flavescens* such as are shown in Pl. xiii., fig. 127, E, F, G ($\times$ 375). The one on the left seems altogether immature, showing a distinct nucleus in each segment and only a very small amount of endochrome; while in two of the others (F, G), found apart from the weed, and only a little larger, the developed form is shown in which the nuclei are more hidden by stationary and a few swarming granules.

The fact of the comparative absence of baby forms of Desmids, of the comparative uniformity in size of the different representatives of the same species, together with the frequently undifferentiated condition of the full-sized specimens, has always seemed to me strongly suggestive of the possibility of Desmids owning a mode of origin apart from, and additional to, their generally acknowledged modes of increase. Why should one find so many of these Desmids of full size and yet with an undifferentiated structure? Surely if they had developed from small specimens produced within zygospores (themselves so rarely seen) they ought also as they increased in size, like other organisms, gradually to assume their typical internal structure? If, on the other hand, Desmids also took origin by processes of transformation from other matrices an explanation of some of these difficulties might be found. Still my occasional search during the last few years for evidence of any such heterogenetic origin of Desmids remained fruitless until quite recently.

During last autumn, as detailed in the last section, I made out the heterogenetic origin of Diatoms from cells of Chlorochytrium in *Lemna gibba* and *L. minor*. I subsequently ascertained that very similar transformations were also to be found with the

[1] Their movements continued while the photographs were being taken, so that no individual granules are represented.

cells of another species of this parasitic alga growing in *L. trisulca*. While working with a dead leaf of this latter plant I was surprised to find one day within one of the Chlorochytrium spaces, and almost filling one-third of it, a large and distinct Desmid, which, as I have since ascertained, belongs to the genus Calocylindrus. This is the specimen shown in Fig. 128, F (× 250).

I soon recognised that it had exactly the same colour and the same kind of granules in its interior as are to be found in certain large cells occurring, often singly, within the capsular spaces of *Lemna trisulca* in which aggregates of Chlorochytrium are contained. Such cells are never to be found in association with the species of Chlorochytrium that infests *L. gibba* and *L. minor*—even when taken from the same pond as the *L. trisulca*. These cells are in fact very much like those figured by Cohn,[1] and as to which he hazarded the suggestion that they might be "resting cells" of the Chlorochytrium.

I have found in some of the dead leaves of *L. trisulca* that one, or rarely more than two, of such cells are to be found in many of the sub-epidermal spaces containing Chlorochytrium, and that they are undoubtedly formed from cells of this Alga which become or remain comparatively large, assume a darker colour than the others, and also become more and more filled with blackish, or brownish-black, motionless granules. Such cells are shown in Pl. xiii., fig. 128, A (× 375) in which comparatively few of the dark granules exist in their interior. Two of them are to be seen contained in small spaces near the broken edge of a leaf. In B (× 375) a larger space (partly empty) is shown containing five of these large dark-granule cells. Two of them are very large and have thick walls; the other three are smaller, and show different stages of repletion with dark granules—the upper one of the three being much more densely packed with such granules than either of the other two.

I found the specimen of Calocylindrus in one of the spaces on November 5 last, and as there were many of the large dark-granule cells in different spaces of this same leaf I put it alone into a small vessel with some fresh water, and kept it near a window under a large bell-jar—carefully examining the leaf at intervals of one to three weeks, and adding a little distilled water to the vessel from time to time to compensate for evaporation.

The cold, dull weather was very unfavourable for the development of these cells; and so, doubtless, was their confinement to a small quantity of water kept indoors and beneath a bell-jar. Still, for a few weeks development did go on slowly; then it

[1] *Beiträge zur Biologie der Pflanzen*, 1870, Band I, Heft. 2, taf. II., fig. 5, k.

became slower still, and by the end of January, after ten days of frost and fog, the vitality of the Algæ seemed reduced to the lowest level, so that they were no longer submitted to observation.

Some of the points that I made out were as follows: These dark granule cells, even after the first month, continued to grow and change slowly, as may be seen from Fig. 128, C, D, E, (× 250). These are three photographs of the same two cells, taken respectively on November 30, January 3, and January 24. The cells were contained on the broken edge of the leaf, and one side of the space in which they had been contained was also broken away. D shows that after five weeks each of the cells had increased in size, and that one of them (in part out of focus) was becoming somewhat elongated. In E, after another three weeks, the cells were only a trifle larger, but the elongated one (somewhat foreshortened in the photograph) had become slightly constricted near the middle—thus tending towards the shape of the Calocylindrus seen in F, which, as I have said, was found actually within one of the spaces, together with small Chlorochytrium segments and one of the dark-granule cells—the latter situated below but in a deeper plane, and therefore out of focus. The colour and the character of the granules within the large cells were exactly similar to those found in the Desmid. Some of the granules in each half of the latter were exhibiting the usual dancing movements, and in some of the large cells dancing granules are also to be seen.

At the date when the first Desmid was found within the space (November 5) I also found, in and near a broken edge of the leaf, four or five other of these Desmids, more or less developed, such as may be seen in Pl. xiii., fig. 129, A (× 125), under a low power. Two of them near the centre are inclined at different angles. In several of the contiguous spaces there were large, spherical, dark-granule cells. The leaf was then put away, as I have said, in a small vessel containing a shallow stratum of water. It was examined again on November 13, and then I found the mature Desmid which is shown in B (× 250). After most careful scrutiny no other specimen was found, and the leaf was restored to the water from which it had been taken. On November 30 it was examined again, and then on the surface of the leaf, near its edge, the immature Desmid shown in C (× 250) was found. This Desmid was certainly not there, as such, when this portion of the leaf was most carefully examined on the date last mentioned. It had been, I believe, developed in the interval from one of the dark-granule cells. It seemed obviously immature: it was rather smaller than the last specimen and less densely packed with granules. A nucleus was distinct in each half, and some of the granules showed dancing movements. Later on, December 5, a

mature Desmid was found, also on the edge of the leaf, which I believe to be the developed form of the specimen last referred to. It is shown under a higher magnification in D (× 375), and a kind of crenation of the edge of the endochrome is distinctly to be seen within its capsule. A nucleus and some dancing granules were also recognised in each segment.

The foregoing observations are of such a kind as to admit of little doubt. They constitute a distinct proof of the heterogenetic origin of a large Desmid, and will doubtless lead before long to the discovery of other analagous transformations, that will suffice to clear up some of the difficulties already cited in regard to the frequent appearance of Desmids, which though nearly or actually of full size have nevertheless a comparatively undifferentiated structure. Observations of this kind would throw light also upon many other points previously obscure, touching some of the sites in which Desmids are to be found, and the geographical distribution of these organisms.

From the point of view of site I have quite recently made a most curious observation. During the examination of a specimen of the ivy-leaf Duckweed I found the three small Cosmaria close together within the substance of the leaf which are represented in Pl. xiii., fig. 129, E (× 250).[1] As the epithelium was quite intact on the upper surface of the leaf I at first thought that the Desmids must be on its under surface. But when it was carefully turned over I found that these organisms were really within the substance of the leaf, and that the epidermis on its under surface was as sound as it was on the upper surface. How could these Cosmaria have got into such a situation? No stomata ever exist in the thallus of this Duckweed; and even if they did, and if germs of Cosmaria were as common as they are really rare, how could such non-motile germs get through them into the substance of the leaf?

Two brief quotations will suffice to make known the nature of some other difficulties of a related order. Ralfe says (*loc. cit.*, p. 14), "The production of Desmids in newly formed collections of water is involved in obscurity. The late Mr. Miller of Penzance pointed out to me an instance of this kind well worthy of notice. He found *Hyalotheca dissiliens* and other species of this family in an old water-butt which stood in a yard remote from any apparent station for the Desmidiæ, and derived its water from the clouds alone; and the question naturally arises, How

[1] The dark body to the left of them was a brown Arcella. In the corner below it, in a slightly deeper plane (and therefore out of focus), there was another Cosmaria—so that there were really four of these organisms in the substance of the leaf.

came the Algæ there?" Again Wolle, speaking of Desmids (*loc. cit.*, p. 15), says, "In New Jersey varieties have been discovered which previously were thought to belong exclusively to the hottest parts of South America; and in the same state are also found species peculiar to Nova Zembla and Spitzbergen; we may assume these latter to be a northern legacy to New Jersey upon the breaking up of what is known as the Glacial period, but we have no plausible reason to give for the presence in the same localities of species which are indigenous to Brazil and the East Indies."

But when it is once ascertained that Desmids may originate by Heterogenesis, explanation of such facts becomes easier. We are no longer tied down to continuous lines of descent, but may have fresh origins of Desmidiæ occurring from more universally distributed common algoid cells, and the subsequent perpetuation of such forms after the manner of Desmids generally.

XIII. ON THE HETEROGENETIC ORIGIN OF EUGLENÆ AND ALSO OF DIATOMS FROM OTHER ALGOID CELLS.

During the latter part of the time that I was investigating the origin of Diatoms from Chlorochytrium segments I recognised that in *Lemna minor* and also in *L. gibba* some of their sub-stomatal spaces were often packed with algoid elements of quite a different kind.

The patches of Chlorochytrium are mostly of a rather pale yellowish-green colour, and its constituent cells are globular, seemingly hollow, having no distinct nucleus and no granular contents: they contain instead variously arranged specks and narrow bands of green endochrome.

The new patches, however, were of a dark grass-green colour, and, where the cells were not too densely packed, it could be plainly seen that each one had a rather large and distinct nucleus surrounded by a finely granular endochrome. These cells, like those of Chlorochytrium, varied much in size owing to their undergoing different degrees of fission in different patches and also within the same patch. None of the new cells, however, in the sub-stomatal spaces were anything like so large as some cells of Chlorochytrium, when it happens that only a few of these latter occupy a sub-stomatal space. In the new Alga fission occurs too early and the subsequent growth of the segments does not permit of their attaining such dimensions.

When densely packed in the spaces the new cells often presented a characteristic tessellated appearance, such as is shown in Pl. xiii., fig. 130, A, B (× 375), though this appearance is never to be seen with Chlorochytrium.

The cells tend to infiltrate the leaf contiguous to the spaces also, and even rather more extensively than is the case with Chlorochytrium. Indications of this are to be seen in A, but much more plainly and extensively in C (× 125) where a larger area is shown under a lower magnification.

It soon became evident that some of these new cells after a time undergo distinct changes within the sub-stomatal spaces. The endochrome becomes a trifle paler, the cell assumes a more ovoid shape, the nucleus remains distinct, and a minute red pigment speck appears near one extremity where there is a slight parting of the endochrome. These bodies then slowly increase in size, still remaining motionless, and the endochrome sometimes assumes a lateral disposition. The units at this stage are shown in D (× 375), pretty closely packed within a space, and also by its side in another partly empty space.

They remain in a motionless condition for a long time (my observations being made in the winter months) but occasionally, during examination and after a prolonged exposure of a leaf under the microscope to light and heat, some of them develop a flagellum and move about as small but typical Euglenæ. I have also seen a number of them swarm out through the dilated stoma of a space when a drop of a dilute osmic acid solution has been passed under the cover-glass, and before they succumbed to its influence.

Two or three weeks later I found other specimens notably larger. They are shown in Pl. xiii., fig. 130a, E (× 250), and appear quite as large as those seen in Fig. 130, D (× 375). The Euglenæ were, as before, motionless, though none of them were encysted. A sub-stomatal space, packed with these new algoid cells, is to be seen by their side and above.

There is no room for the slightest doubt that it is these algoid cells which undergo the apparently slight changes that I have mentioned—the alteration in tint and shape, together with the formation of a minute red eye-speck—by which they become converted into embryo Euglenæ. The ultimate molecular alterations that must occur in order that this transformation may be brought about are, of course, altogether beyond our ken.

I am far from supposing that what I have described above is the only heterogenetic mode of origin of Euglenæ, though it is evidently a very common one, and one whose stages can be recognised with the greatest ease; not of course on the same specimen, but in different specimens on the same dead thallus of a Duckweed on which the parent epiphyte happens to be abundant.

As bearing upon this point the following circumstance may be mentioned. A large pond at Northwood during last summer and autumn was mantled over with the three kinds of Duckweed; the ivy-leaf Duckweed being confined to about one-sixth of the

pond and at one extremity only. I chanced to see this pond again during the first week in November after an interval of about a fortnight, during which there had been some frosty nights, when, much to my surprise, I found almost all the *Lemna gibba* and *L. minor* gone or dead, and the parts of the pond where they had been were now pretty thickly covered with a green scum of what seemed to be Euglenæ, and which on examination proved to be of this nature. The *Lemna trisulca* was also dead, but over this part of the pond there was no mantle of Euglenæ.

I was much puzzled at first to make out the nature and source of these new products, but I soon found that they were derivatives of a small epiphyte found not infrequently on the upper and lower surfaces of both the common Duckweeds—that is, upon dead and decolourised leaves, and mostly in association with Chlorochytrium. I have indeed occasionally found both these Algæ within the same sub-stomatal spaces — the new Algæ generally above and adding themselves, as joint tenants, to the Chlorochytrium cells.

On the surface of the leaf the new Alga appears as small, flat, distinctly nucleated cells, varying much in size according as segmentation had or had not taken place as growth progressed, as may be seen in Pl. xiii., fig. 131, A (× 375). Like the cells contained in the sub-stomatal spaces, they show around the distinct nucleus a finely granular green endochrome. Minute cells of the same kind were also to be seen occasionally multiplying within an epithelial cell—just as with Chlorochytrium. And in these cells, as well as in the sub-stomatal spaces, the segments of the Alga seem at times, instead of changing into Euglenæ, to undergo conversion into Diatoms of the Navicula type, not very different from those that are produced from Chlorochytrium segments.

It is quite impossible to say what are the conditions that favour the transformation of these cells into Euglenæ rather than into Diatoms, or indeed why either of these transformations should occur, seeing that in the same Duckweed thallus, aggregates of the Algoid cells may be seen multiplying as such side by side with others undergoing conversion into Diatoms and, here and there, others still into Euglenæ.

The evidence for the conversion of the cells into Diatoms is exactly of the same kind and just as strong as that which I have adduced in regard to Chlorochytrium. It will not be necessary therefore to dwell upon this change at any length. In Pl. xiii., fig. 130a, A (× 375) a space is shown in *Lemna minor* containing many of the large nucleated cells, intermixed among which there are a few small Diatoms. In B (× 375) the Algoid cells were fewer, and their place was taken by a much larger number of

Diatoms; while in c (× 375) the Alga was represented only by smaller segments and the brownish-yellow Diatoms were densely packed among them. Then, again, as I have already stated, the cells of this Alga are often found infiltrating themselves extensively through intercellular spaces contiguous to one or more of the patches, such as I have shown in Fig. 130, c; while at other times, in precisely similar situations, we may find such intercellular spaces packed in the main with minute Diatoms, as was the case in the area shown in Fig. 130a, D (× 125). Finally in these situations, in portion of the sub-stomatal spaces, as well as in the epithelial cells, the actual transformation—that is, all stages of it—can be plainly made out. The green Algoid cells may be seen elongating and taking on the brownish-yellow colour and appearance of the Diatoms. These have been mostly small and of the Navicula type; no Nitschiæ having ever been seen to be produced from the cells of this Alga.

The growth of the Alga as an Epiphyte has still to be referred to.

Large superficial groups of cells like that represented in Pl. xiii., fig. 131, A (× 375), are not at all common; the cells are much more frequently distributed over the leaf either singly or in groups of twos or threes. In B (× 250) several of these cells are to be seen within a sub-stomatal space; and even though the magnification is low their nuclei and finely granular texture can be distinguished.

On the under surface of the leaf, more especially, many of the cells germinate, and send upwards a short filament, not often, so far as I have seen, of more than five or six segments. Such cells may be found side by side with upward projections consisting of one, two, three or more segments as in Fig. 131, c (× 375). When the parent cells are about to germinate in this way a number of small, fatty-looking particles appear around the nucleus[1]. The terminal segment after a time seems to resolve itself into a brood of microspores. I have not been able to follow this process clearly, but I have several times seen within a terminal segment the green endochrome gone and a number of very minute almost colourless zoospores, sometimes free and sometimes contained within a very delicate hyaline envelope. It looks, therefore, as if the endochrome gathers into a central mass, and then becomes in great part decolourised and resolved into minute zoospores. The process probably takes place rapidly. I have not yet succeeded in tracing the different stages, though

[1] The cell itself is occasionally found to be surrounded with a rust-coloured border, such as is very commonly seen around the germinal cell of Algoid epiphytes.

it is extremely common to find this little epiphyte with its terminal segment empty—or even sometimes, when there is only a single segment, to find that empty and open at its free extremity.

Since writing the last sentence I have found the specimen, a representation of which is given in Fig. 131, D (× 250). It shows the terminal cell, yielding zoospores, of a very similar, though rather larger epiphyte, also met with, though rather sparingly, on the leaves of Duckweed.

I am unaware of the name of the little epiphyte whose different states I have just been describing. Nothing like it is to be found described in the works of Hassell or of Cooke on "British Fresh Water Algæ," nor in Wood's memoir on "The Fresh Water Algæ of the United States." It has been suggested to me that it is a small Œdogonium, but no such formation of minute zoospores as I have described has been observed in members of this genus.

Before leaving this subject of the heterogenetic changes to be met with in the different species of Duckweed I may briefly allude to what I believe to be two others which are often recognisable in the leaves of the two common Duckweeds when they are in a dying state and almost decolourised. It is extremely frequent in such leaves to find within the epithelial cells, and also within the deeper spherical cells, a number of minute, oat-shaped Monads—perhaps two or three in each of the former cells happening to contain them, and six to eight in the deeper cells, mixed with a little granular refuse matter, as in Pl. xiii., fig. 132, A, B (× 375). Allowing the cells to soak for a few hours in a very weak dilution of Westphal's "mastzellen" stain,[1] one finds that the chlorophyll corpuscles that are only beginning to decolourise in some of the cells do not become stained; other of the corpuscles, however, more completely decolourised and containing fine granules in their interior take up the stain, though not so deeply as any Monads which may be associated with them. Some of such differences in tint may be seen in B. Whether this be the mode of origin of the Monads or not, there remains the fact that multitudes of the closed spherical cells within the substance of some leaves may be found, on careful examination, to contain these minute Monads—whose entry from without, simultaneously and in such numbers, into contiguous cells seems scarcely conceivable. It is all the less conceivable because such Monads are scarcely ever to be seen outside the cells.

[1] I keep this stain diluted with a two per cent. formalin solution, as with water alone Torulæ and a minute Mould are apt to appear in it after some weeks.

The other change is generally associated with that to which I have just referred, but it is one that occurs in a much smaller number of the cells. Here, instead of Monads, we find in the affected cells one, or perhaps two, very sluggish nucleated Amœbæ, such as are shown in C (× 375), and almost all the granular contents of the cells gone. This may be only one of the later stages prone to occur in the cells in the condition previously described—due to one or two of the Monads assuming an Amœboid phase, and then gradually devouring everything else within the cell in which they are found.

Observations of a somewhat similar nature have been described in detail by Braxton Hicks.[1] He saw the chlorophyll corpuscles of certain Moss radicles notably increase in size and become cellular, while their contents divided into three, four, or more motionless segments. The modified corpuscles remained for some months in this condition; though after this time when some of them were exposed to sunlight for an hour, on a glass slip and beneath a cover-glass, the contained segments seemed to be rapidly converted into very active, faintly green Monads.

XIV. ON THE POLYMORPHISM AND HETEROGENETIC ORIGIN OF SOME FRESH WATER ALGÆ.

Many of the classificatory distinctions adopted by systematic writers on Fresh Water Algæ are made to depend upon the most trivial differences. Mere slight variations in colour of cell-contents, for instance, have caused forms otherwise presenting the greatest similarity to be altogether separated and ranged in distinct classes. No more notable instance of this can be found than the separation of the numerous genera and species belonging to the families Palmellaceæ and Protococcaceæ from many others included in the family Chroococcaceæ, simply because the cells of the latter mostly possess a bluish-green rather than a bright grass-green colour.[2] Let any one compare the figures in the numerous plates given by Cooke in his "British Fresh Water Algæ" illustrating the species belonging to these different families, and he cannot fail to be struck with their extreme similarity. Cooke has himself called attention to this relationship, and to the memoirs of P. Richter in reference thereto. He quotes (p. 2)

[1] *Quart. Journ. of Micros. Sc.*, April, 1862.

[2] Pringsheim has shown that the application of heat alone will bring about these as well as other modifications of colour frequently to be met with during heterogenetic transformations. Speaking of green leaves boiled in water for five minutes, and of the drops of colouring matter which may then be seen exuding from the chlorophyll corpuscles, he says: "Always coloured, they are usually chlorophyll-green, but this may be brighter or darker, and the tint in some cases is yellow or blue-green, occasionally olive-green, more seldom reddish-brown"—(*Quart. Journ. of Micros. Sc.*, 1882, p. 77.)

the following conclusions arrived at by this investigator:—"The lowest form of the Phycochromaceæ is the naked *Aphanocapsa* condition, corresponding to *Palmella* among the Chlorophyllophyceæ. From this naked or only slightly encysted condition is developed the *Glæocapsa* or *Glæocystis* form with several gelatinous envelopes; the Chroococcus type, when the investment is altogether wanting; or, when there is only a slight vesicular envelope, the cænobium types. The *Glæocapsa* type is specially adapted for exposure to the air, and growth upon a comparatively dry substratum; the Cænobium type is developed in water; the *Chroococcus* type in water, or on a moist substratum in the air. With this is connected the cylindrical form, a higher stage, because it displays a differentiation in the direction of growth, and a development towards the filiform condition." He also quotes Richter's account of the various "successive conditions," forwards and backwards, which some of these forms may pass through.

Braxton Hicks[1] had previously shown the close relationship and convertibility under different conditions of various species of Hæmatococcus, Coccochloris and Sorospora—he showed, in fact, that by slight variations of the environment there may be produced no less than twenty-three forms which had hitherto been regarded as distinct species of fresh-water Algæ.

After his brief description of the family Chroococcaceæ Cooke says (*loc. cit.*, p. 203):—"It may be urged that as many of the species included in this family, as well as in the analogous *Palmellaceæ*, are only conditions of higher forms, they should not have been inserted. In the preparation of a Flora of this kind, however, we are of opinion that whilst the life-history of these forms is so imperfectly known, we should not have been justified in excluding them." That may in a measure justify his description and portrayal of them as so many separate species. On the other hand, it looks as if a more perfectly known life-history, under changing conditions, of these most plastic forms of life, might only suffice to reveal still other modifications and interrelationships. Braxton Hicks says, "I believe we shall be obliged to conclude that all the cells classed as Palmellaceæ—*Chlorococcus*, *Glæocapsa*, *Sorospora*, and some others, with their so-called species—are but varieties of one mode of simple vegetative cell-growth, common to most Cryptogamia." He makes this statement because he thinks it "impossible to discriminate between the cells of the segmenting gonidia of Algæ, of Lichens, and of Mosses." Then again we are told in the last edition of Carpenter on "The Microscope,"[2] that "the *Palmellaceæ* are not

[1] *Quart. Journ. of Micros. Sc.*, 1860.
[2] Eighth Edition, edited by Dr. Dallinger, 1901, pp. 559 and 545.

now regarded by the best authorities as a distinct family from the *Protococcaceæ;*" and in regard to the type of this latter family it is said, "To what extent *Protococcus* is an autonomous organism is still doubtful, but it appears to be more or less closely connected with many forms of life which have been described, not merely as distinct *species*, but as distinct *genera* of animalcules or of protophytes, such as *Chlamydomonas, Euglena, Trachelomonas, Gyges, Gonium, Pandorina, Botryocystis, Uvella, Syncrypta, Monas, Astasia, Bodo,* and many others."

But to a lesser extent the same kind of polymorphism that obtains among these unicellular Algæ is also to be met with among higher filamentous forms. Thus Cooke writes (p. 183) :—"In 1861 Dr. Braxton Hicks indicated his belief that *Schizogonium* was only a condition of *Ulothrix* in which the threads had become connate, of which *Prasiola* was only a frondose form. He says, 'The whole of these changes are so palpable, can be observed so constantly, and are, at the same time, so simple in their relations to one another, that one can scarcely imagine how they can have been separated, not only into distinct species, but into different families of Algæ. Thus the linear stage is called *Lyngbya* (Ulothrix); the early stage of collateral segmentation, the *Schizogonium;* the adult stage the *Prasiola;* while the gonidial growth has been classed under *Palmellaceæ.*'" Dr. Cooke states that the whole of the communication[1] whence the above is quoted is "worthy of attentive perusal."

It is then from plastic forms of growth of this kind that the transformations described in the preceding three sections have been produced. We have seen cells of simple Algæ becoming transformed into other aberrant types of Algæ—that is, into Diatoms and Desmids—and also into Euglenæ. These latter, belonging to the Phytozoa, have, however, close relationships with some Algæ through the genera Chlamydococcus and Chlamydomonas belonging to the *Volvocineæ.*

The question that now presents itself is, may Algæ themselves have a heterogenetic mode of origin? And this question I am able to answer in the affirmative, as may be seen from the following subsections.

(*a*) On the Origin of Vaucheria from Encysted Euglenæ.

One of the most remarkable observations that I have made on this subject revealed the origin of Vaucheria filaments from encysted Euglenæ. These observations were made on a Euglena pellicle which had been under daily observation for nearly three

[1] *Loc. cit.*, 1861, pp. 157-166.

weeks—the stock of Euglenæ being that referred to on p. 12 as having been taken from the surface of a small lake at Loughton. During the previous daily observations no trace either of Vaucheria filaments, their resting spores, or zoospores had been seen. The pellicle was almost spent, owing to changes of different kinds which had been going on in it, and the few Euglenæ that remained were almost all encysted. Many of the cysts, as is commonly the case in old pellicles, had assumed a distinctly brown colour.

My surprise was great one evening, when a portion of this pellicle was under examination, to find the Vaucheria filament, which is shown in Pl. xiii., fig. 133, A (× 150), emerging from one of these brown cysts. The filament, as it emerged, had almost exactly the same breadth as the cyst. Only a very few scattered chlorophyll corpuscles were to be seen on its wall, near the cyst, as I have seen not unfrequently in filaments issuing from Vaucheria resting-spores; then came a slanting dissepiment such as is also often to be seen in Vaucheria filaments; immediately beyond it there were still very few chlorophyll corpuscles, though towards the extremity of this short filament they became much more numerous, indications of which are recognisable in the figure. The brown cyst was seen to be split open where it gave exit to the Vaucheria filament —and as to its being a Vaucheria filament there is not the smallest room for doubt I have worked, off and on, for years with this weed under various conditions, and could not possibly fail to recognise a Vaucheria filament.

During this evening and the next I found four other specimens precisely similar except for variations in the length of the filaments. It was clear that the Vaucheria tube in each case issued from one of the brown Euglena cysts. It seemed also to have been formed in each case from the undivided Euglena contained within the cyst. These particular cysts were in size and colour like the other Euglena cysts with which they were associated, and they bore no sort of resemblance to the only two reproductive elements pertaining to Vaucheria. This may be seen by reference to C (× 150) showing Vaucheria resting-spores under the same magnification, and to B (× 70) showing at less than half the enlargement a zoospore about to be formed from one of the filaments. In regard to the comparative absence of chlorophyll corpuscles in the young filaments proceeding from these cysts, I may say that I have several times seen the same kind of thing in the young filaments produced by the germination of one of the resting-spores when they have been kept for some time under unfavourable conditions.

On no other occasion have I ever met with anything of this kind. I am reminded, however, of what I think may often be

seen, and that is Vaucheria or Oscillatoriæ growing in a partially dried up ditch where Euglenæ had previously existed. Of course it is impossible to say whether this succession is due to mere chance, or to the existence of some causal or genetic relationship. Still, what has gone before makes this particular succession worthy of note.

(*b*) On the Origin of *Microspora fugacissima* and *Scenedesmus acutus* from the Corpuscles of small Euglenæ.

The transformation last described took place in an old Euglena pellicle, and therefore with free exposure to air; but that to which I am now about to refer occurred in Euglenæ which had sunk to the bottom of a small beaker containing clear water to which portions of a Euglena pellicle had been transferred. This change, moreover, was observed going on for some time—that is week after week—and many specimens were submitted to careful examination. They were first seen about six weeks after the Euglenæ had been transferred to the beaker, which had been kept in the meantime on the mantelpiece under a glass shade. On examining the vessel with a lens one day I found a sparse green deposit at the bottom, as well as on the sides, of the small beaker, mixed with some minute algoid filaments. This induced me to submit some of the deposit to examination.

I found the Euglenæ in motionless aggregates, though not encysted. They were all of a dark grass-green colour, with a minute red eye-speck in each. They were filled with round or ovoid green corpuscles, and in places the superficial envelope of the Euglenæ was softening and the enclosed corpuscles were being liberated, as may be seen in two of them represented in Pl. xiii., fig. 134, A (× 375). When free these corpuscles grow and multiply as independent algoid elements, such as may be seen in A and also in all stages in B (× 375). Some of them develop into the filaments which are shown beginning in B, and more completely developed in A, where they may be seen to present the characters of *Microspora fugacissima* as given by Cooke in Pl. liii., fig. 1.

Repeated examinations, as I have said, left no doubt as to the reality of these transformations. It will be observed in A as well as in B that some of the cells are distinctly nucleated and have a pointed ovoid shape, and these cells multiplied in such a way as to produce groups of four, as shown in C (× 250), having almost exactly the characters of *Scenedesmus acutus* as given by Cooke in his Pl. xiii., fig. 6. Numbers of these groups were seen with all the patches of transforming Euglenæ. Yet this genus *Scenedesmus* was considered by Ralfe, and also by Hassall, to belong to the Desmidiæ.

(*c*) On the Origin of a Minute Conferva from the corpuscles of a small Euglena.

The changes now to be described occurred under conditions very similar to those last referred to. Portions of a Euglena pellicle containing encysted Stylonychiæ and Rotifer resting eggs with double envelopes which I was studying, were transferred to the surface of fresh water in a tall vase. After about four months portions of the sediment which had collected at the bottom of the vase (as referred to on p. 34) was repeatedly examined, and, among other things, I found therein a number of small Euglenæ, again motionless but not encysted. Each of them showed a small red pigment speck, and their bodies were densely packed with very minute dark green corpuscles. These small Euglenæ were mostly found singly or in very small groups scattered through the sediment. Here and there their envelopes were undergoing softening, and the minute green corpuscles with which they were filled were being liberated. The corpuscles then seemed to multiply independently, and some of them grew into minute, dark green, rosary-like threads. In D (× 375) two of these Euglenæ are shown in close contact, and the envelope of the upper one has given way, and is giving exit (on the right and above) to the minute green corpuscles. A small group of these corpuscles is also to be seen in which they are undergoing segmentation, while to the right some of them are growing into monilated threads. In E (× 500) one of these threads is shown more highly magnified. It seems to be a very minute Conferva, though I can find nothing like it represented in the works either of Cooke or of Hassell.

Some of the sediment containing these Euglenæ was subsequently transferred to fresh water in a small tube, which was corked and left on my work table. And for several weeks after, on examination from time to time, I saw the same kind of change going on. The minute Conferva corpuscles and threads occurred in small separate heaps, and almost always in immediate relation with broken-down Euglenæ. In some few places, however, the Euglenæ were ultimately either obscured by, or completely converted into, such aggregates.

(*d*) On the Origin of *Nostoceæ* within some Gonidia from Mosses.

During my examination of the specimens of Lichen (*Parmelia parietina*[1]) brought from the Pyrenees (see p. 114), I have found that portions of it were mixed with small sprigs of Moss; while other portions, gathered elsewhere (at Luz and St. Sauveur), were free from such admixture. From the former

[1] I find this Lichen is more commonly known now as *Physcia parietina*.

specimens, but not from the latter, examined in the way I have described, I have frequently found small Moss gonidia like those shown in Pl. xiii., fig. 135, A, B (× 250). The capsules of the smaller specimens such as are represented by A are almost colourless, but when larger, as in B, and larger still, they have a distinctly yellow or brownish tint, the chlorophyll corpuscles within being green. These gonidia were found of different sizes, and still larger bodies, up to $\frac{1}{200}$ in. in diameter, in exactly the same kind of capsules were met with in which the contents were of a blue-green, and were seen to be composed of a dense aggregate of moniliform threads, such as are shown in C and D (× 250). The capsule is not seen in D, because the upper surface of the sphere was focussed in order to show the threads more clearly. A portion of one of these threads from a crushed capsule is represented under a higher magnification in E (× 375). There was certainly no jelly-like material with the threads within the capsule, so that, as Dr. Cooke suggested to me, they probably belonged to the genus Anabena rather than to Nostoc.

The similarity between the capsules in the large and the small aggregates was so exact as to make it seem highly probable that the large were only later stages of, or derivatives from, the smaller spheres, and this was subsequently proved. One of the smaller specimens was kept, still beneath the cover glass, in a damp chamber for a fortnight, and during this time it increased greatly in size and showed, especially at one border, moniliform threads forming. The capsule was yellow as usual. It is always a tough membrane, and requires a very distinct amount of pressure to rupture it, as I found when procuring E for examination from another specimen.

Algæ of a different kind, but belonging to a closely allied family, have been seen to be derived from the leaves of Mosses by Braxton Hicks.[1] He says he has frequently seen masses of Glæocapsa developed from the older leaves at the base of the stem of many Mosses. These leaves frequently assume a brownish aspect in winter and spring, owing to the cell walls taking on this colour, though their contents still remain green. But, as this observer says, "After a while the old cell-wall dissolves away, and then it becomes evident that the contents have assumed the form of, or rather have become, a Glæocapsa, which certainly undergoes segmentation freely. . . . . I have seen considerable masses of Glæocapsa produced in this manner."

It will be observed that the walls of the gonidial cells observed by me had also taken a yellow or brownish tint, such as B. Hicks has referred to as occurring in the cells of the Moss leaves.

[1] *Trans. of Linnean Soc.*, 1862, p. 581, Pl. lviii., fig. 24.

(*e*) On the Origin of *Anabena* from the Chlorophyll Corpuscles of *Lemna trisulca.*

An isolated, decolourised leaflet of *Lemna trisulca* was found in which, along the centre and towards its proximal extremity, green chlorophyll corpuscles were still to be seen lining the spherical, sub-epidermal cells. In two separate lateral regions of this leaflet ill-defined patches were found in which the chlorophyll corpuscles were mostly decolourised and showed only a few colourless granules in their interior. Intermixed among them, however, there were other chlorophyll corpuscles which still preserved their green colour. A portion of one of these patches is shown under a low power in Fig. 136, A (× 250).

As I wished to see what further changes these corpuscles would undergo, I placed this leaflet alone in a small tube with some fresh water, and left it under a bell glass on my mantelpiece. Three weeks later I examined the leaflet again, and found, in the same patch, that some of the corpuscles were still single and colourless, while others of them were of a pale blue-green, or of a pale blackish-purple, and intermediate conditions were also recognisable. Those that were coloured in the way I have indicated were either single, or had grown into chaplets of three, four, or more elements, such as are shown in B (× 500). The same kind of changes were going on in the other patch of decolourised chlorophyll corpuscles. *Yet all of them were contained within closed spherical cells covered by an uninjured epidermis on each side of the leaf.*

For two weeks more I examined this leaflet from time to time, and found the same changes slowly progressing. Soon afterwards they seemed to cease. The continued examinations, as well as the confinement indoors in a small tube beneath a bell-jar, were doubtless not a little unfavourable. Still the trichomes of Anabena grew in length and increased in number, varying somewhat in tint, but being mostly of a pale purplish-black colour.

(*f*) On the Origin of *Anabena* from the Cells of *Chlorochytrium lemnæ.*

In the memoir by Ferdinand Cohn, "Ueber Parasitische Algen,"[1] in which this Chlorochytrium was first described as infesting *Lemna trisulca,* he figures four different kinds of organisms as occasionally to be met with in spaces which had been previously tenanted by Chlorochytrium, namely, a species of Raphidium, of Mastigothrix, of Leptothrix and of Nostoc. He assumed that these were all parasites which had in some way

[1] *Beiträge zur Biologie der Pflanzen,* 1872, p. 97.

obtained entry to the spaces in question. He did not attempt to verify this assumption; he seems to have taken it for granted, in accordance with generally received doctrines, that this was the proper explanation to be given.

I have never met with either of the first three organisms named above in the Chlorochytrium spaces, but I have, on many occasions, seen one of the Nostoceæ; and am prepared to adduce evidence as to its mode of origin within these cavities. I have, however, never seen the slightest evidence in proof of the assumption that they get in from without. Cohn says he found a representative of the genus Nostoc (*N. glomeratum*), but the organism which I have seen is apparently an Anabena. The filaments were free, and certainly not imbedded in any mucilage or jelly-like matter. And in reply to a query of mine Dr. Cooke writes, "filaments without the definite mucilage do not, to my mind, constitute a true and veritable Nostoc. I could only suggest Anabena, with free filaments, as an alternative to Nostoc."

It must be borne in mind, however, that the filaments in each of these genera are endowed only with a minimum amount of mobility, of an oscillating type, and that their spores are *non-motile*; so that the conditions are very unfavourable to their parasitism within closed cavities, or cavities with apertures only of the most minute dimensions.

I have found that specimens of Lemna containing these Algæ, when mounted in a mixture of glycerine and formalin[1] are well preserved, and are in some respects more favourable for examination than when they are simply immersed in water. The colours of the Nostoceæ are preserved extremely well in specimens mounted and preserved in this medium.

Just the same kind of combinations are to be met with as in the case of the transformation of the fission products of this Alga into Diatoms—that is, the Anabena and the Chlorochytrium may be found mixed in the most varied proportions, or the Chlorochytrium may be completely replaced by the Anabena. Moreover, in specimens of the former kind, the individual elements of the Chlorochytrium may often be observed actually undergoing the process of transformation into Anabena, and changing in colour from green to blue, purple or even red. This process of transformation may be seen taking place in a sub-epidermal space which is still densely packed with the fission products of Chlorochytrium—and such transformed elements may be seen presenting themselves either in the centre of the mass or at its periphery. The typical colours of the Anabena show themselves first in corpuscles, while these corpuscles subsequently multiply so as to produce the necklace-like chains.

[1] One part of glycerine to two parts of a 2 per cent. solution of formalin.

In Pl. xiii., fig. 137 (× 375) some illustrations will be found of the foregoing statements; thus A shows a space in which some small and several large green Chlorochytrium segments were found in association with purple Anabena corpuscles; in B a larger space is shown, in which there is a nearly equal and diffused admixture of the green Chlorochytrium together with purple and blue Anabena elements; in C a space is to be seen distended by Anabena chains, which showed under the microscope only three or four minute Chlorochytrium segments remaining; while in D some of the blue Anabena is represented growing free, after having burst through some of the cells at the broken edge of one of the leaflets of the Lemna.

It was supposed by G. Klebs that the *Chlorochytrium Lemnæ* and *C. Knyanum* were only different states of a single species. On this account, therefore, as well as on others, it is of interest to note that the large cells full of dark granules, some of which may develop into Desmids, have never been seen by me in association with the Chlorochytrium that occurs in *Lemna gibba* or *L. minor*, and the same thing has to be said in reference to the presence of Nostoceæ. These, like the last, have only been found associated with the Chlorochytrium growing in the intercellular spaces of *L. trisulca*. It is, of course, possible that the differences I have indicated may be mainly dependent upon differences in the host (the environment), and that G. Klebs may be right in supposing that we have only to do with a single species of Chlorochytrium infesting the three common Duckweeds.

In his memoir on Chlorochytrium reference is made by Cohn to two or three cases in which Nostoc has been found leading a quasi-parasitic life in the tissues of higher plants. In an abstract by Archer[1] of this memoir he writes: "Reinke describes the occurrence of parasitic Nostochaceous algæ in the stems of five species of *Gunnera*. The alga lives at first in the mucus produced from the glands on the back of the young leaves; this mucus infiltrates afterwards amongst the epidermal cells of the *Gunnera*, resolved themselves, too, in great part into mucus, and with it the *clusters of algal* filaments gain an entrance. The passage to the *Nostoc*-clusters becomes closed by newly-formed parenchyma replacing the previous glandular tissue, and the alga is completely imprisoned, so that henceforth its nutriment is obtainable only from the sap of the *Gunnera*." Janczewski has also found a *Nostoc* in the interior of the tissue of certain Liverworts. It penetrates through the stomata on the under side of the thallus of the Liverwort, and thence may spread through its intercellular spaces. The same observer found

[1] *Quart. Journn. of Micr. Sc.*, vol. xiii., 1873, p. 369.

Nostochaceous filaments which had made an entrance through an existing opening into the spiral cells of *Sphagnum*. These cases are, therefore, totally different from those which I have recorded in the last three sub-sections.

XV. ON HETEROGENETIC PROCESSES IN, AND ASSOCIATED WITH, THE DEVELOPMENT OF VAUCHERIA RESTING-SPORES.

Comparatively few persons have probably followed the development of the resting-spores of Vaucheria, owing to the length of time they remain in a dormant condition. I have long been familiar with these bodies, and with various changes that are from time to time apt to occur therein. But until the summer of 1901 I had never seen them germinate and give rise to young Vaucheria plants. The only description of their germination that I have been able to find is that given by Pringsheim.[1] He says: "The spore remains for some time longer, without being thrown off from the parent tube on which it was produced; but the colour of its contents, which was at first green, gradually becomes paler and paler; the spore is at last rendered quite colourless, and presents, in its interior only, one or more largish dark brown bodies. When it has lost all its colour it is detached from the parent tube, in consequence of the decay of the membrane of the sporangium enclosing it. After some time (in my experiments after about three months) the spore, which is readily recognisable by the red-brown nuclei in its interior, suddenly resumes its green colour, and immediately thereupon grows into a young Vaucheria, exactly resembling the parent plant. Close observation shows that the innermost layer, elongating, breaks through the thick outer membrane and becomes the young tube, exactly in the same way as I have described the process of development in the germinating spore of Spirogyra."

On May 8, 1901, I had under examination a quantity of one of the larger Vaucheria which had been gathered a fortnight previously, and placed with water in a shallow dish. Much of the weed had died, but on and among the filaments I found a very large number of resting-spores. A quantity of these were, on this day, placed in a small wide-mouthed bottle, loosely covered with a screw cap, merely to exclude dust and diminish the amount of evaporation. The bottle was half filled with water, and was then left, not far from a window, on the end of a mantelpiece in my study.

[1] "On the Impregnation and Germination of Algæ." Translation in *Quart. Journ. of Microscop. Science*, 1856, p. 63, Pl. iii., figs. 17—20. The figures, as reproduced in the Journal and as copied in Cooke's "British Fresh Water Algæ," are very crude and even erroneous in several respects—this is especially the case with Fig. 17.

The bottle was at first opened only on two or three occasions for a brief examination of its contents. The spores were soon found to be undergoing the common kind of change—that is, were becoming decolourised into a whitish-grey mass of granules and vesicles, containing in its midst from one—four aggregations of pigment granules. The pigment heaps in this case were of a brownish-red or red-orange colour, though very frequently the tint is found to be of a blackish-green.

After an interval of several weeks (on July 4), I examined the contents of this bottle again, and in the first portion of the deposit taken up with a pipette I found a number of the resting-spores germinating and giving birth to filaments. They were associated with other spores in their ordinary condition, and others still in which different changes had been taking place.

Both the resting-spores themselves and the filaments that had grown from them were lined, sometimes pretty thickly and at others very sparsely, with bright green chlorophyll corpuscles. In regard to the filaments, the most common arrangement was that the single process sent out almost immediately divided into two at a very obtuse angle; at other times the division took place at some distance from the spore; while occasionally two filaments were seen coming off from the spore itself close to one another. Subsequently they branched and changed in diameter in a very irregular manner.

The most remarkable facts, however, about these germinating resting-spores have had reference to the contained heaps of pigment—loosely spoken of in the foregoing translation from Pringsheim as "nuclei." The facts observed were so remarkable that I was anxious to repeat my observations in the following year, and this was done.

On June 8, 1902, I found a quantity of *Vaucheria racemosa* growing at the edge of a pond which was absolutely crowded with resting-spores. Some of this plant was kept in water in a shallow dish, and after a day or two numbers of the spores were placed in a small stoppered bottle half full of water. Another large quantity of spores was placed with water in a tumbler, and merely covered loosely so as to exclude dust.

On July 24, that is only six and a half weeks from the date of gathering, many of the resting-spores within the stoppered bottle were found to have germinated; though at that date none of the spores in the tumbler could be found in this condition. Subsequent examinations made it clear that germination took place more rapidly in the closed bottle than in the open tumbler. I have no record of the date when I first found them germinating in the tumbler, but I can say that at the expiration of three and a half months hundreds of these spores were germinating, and that the process was seen occurring in others of them during the next two months.

It may not be out of place to say a few words now concerning the condition of these resting-spores in the long interval that occurs between decolourisation and germination.

For a long time they remain, without apparent change, of a greyish-white colour, owing to the presence of an intimate mixture of colourless granules and corpuscles, the latter seeming to be derivatives or remainders from the green corpuscles with which the spores were originally packed. More or less in the centre a large mass of pigment granules, mostly of a blackish-green colour (Pl. xiii., fig. 138, A, × 250), but sometimes of a red-brown or red-orange tint, as in *V. racemosa*, is to be seen. Three or four, or even more, smaller pigment-heaps, instead of one large one, are very common in this latter species. The largest number I have ever found is shown in B (× 250). It seems perfectly clear that these heaps of finely granular pigment are merely refuse products left over during the process of molecular transformation that the spore has undergone in becoming decolourised. Microscopical examination shows that they are mere heaps of fine granules, unsurrounded by any bounding membrane.

Now comes the question what changes are undergone by the resting-spore in its ordinary condition, such as is shown in Fig. 138, A, previous to its germination. It is very difficult to be certain as to this, but my impression, formed after the examination of very large numbers of these bodies, is that in the normal course of development the corpuscles indicated in A almost completely disappear, and that the general substance of the spore becomes resolved into a uniform mass of very minute granules. The spore has then a rather glistening, silvery-white appearance, such as was seen in B. In the specimen represented in C there was much the same silvery-white appearance, but there were indications that a new set of rather smaller spheres was forming, leading on to the production of small glistening spheres of protoplasm such as are shown in D. Thereafter these small spheres seem to become green and converted into small chlorophyll corpuscles. A specimen of this kind is shown in E, but in an early stage, as the corpuscles were still only of a very pale green colour. Pringsheim speaks of "the innermost layer elongating," and says it "breaks through the thick outer membrane, and becomes the young tube." These two layers are distinguishable in E, which is, I believe, a spore becoming green and just about to germinate; while in Fig. 140, D, the split in the outer membrane is recognisable.

Pringsheim further intimates that these latter changes are rapidly brought about. He says, after a time the spore "suddenly resumes its green colour, and immediately thereupon grows into a young Vaucheria exactly resembling the parent

plant." As to the rapidity of these latter processes my own observations do not enable me to make any definite statements.

I now come to the most interesting and important point of all in connection with the germination of the Vaucheria resting-spore—namely, as to the fate of the pigment heaps which, all along, have been such prominent objects in the resting-spores. During my first examination of these germinating-spores, and on all subsequent occasions, I have found, either in the green spore itself or in one of the filaments issuing therefrom, one or more of the blackish-green, or red-brown pigment heaps now appearing (when not pressed together or squeezed within a filament) as perfect spheres with sharply-defined outlines, such as are shown in Pl. xiii., fig. 139 (× 250). The most surprising thing at first was to see these pigment-spheres in the filaments, as in D and in E. The latter body is only enlarged half as much as the others, in order to show the three unequal pigment masses far away from the spore itself, and jammed together in the filament.[1] Many of the spheres have been seen very much further away in the filaments than this; and many also have been seen just emerging from the spore, as in C, where the middle body is much compressed between the other two.

To casual observation these bodies all appear to be motionless, but, after exposure for a little while to the light and heat from the microscope lamp, very faint and more or less imperceptible movements can be detected in most of them. During my first study of these bodies, while examining a spore containing two of them, as in B, I saw one of the spheres moving forwards and backwards over a space, scarcely equalling its own breadth, without any visible change in shape, and with a slow gliding movement like that of Actinophrys. But through the walls of the spore no rays of any kind could be detected then or since, though I have repeatedly watched their very slow movements taking place when these spheres have been within the spores, and also within the filaments. Sometimes there has been a distinct interval between the forward and the backward movements. At other times it is clear that the movements of the pigment spheres must be more continuous in one direction.

In October last I had under observation a spore in which, within and just outside, there were four unequal pigment spheres, and also two others some distance away within the filament. This specimen is shown in Pl. xiv., fig. 140, A (× 125), though the two spheres close together in the filament are very indistinct owing to their being out of focus. After I had taken this photo-

[1] One of the two smaller bodies is flattened against the upper wall of the filament.

graph, with a short exposure, I noticed that the movements of the spheres within and near the spore were more marked than usual. I left the specimen, therefore, exposed to the light and heat of the lamp (I was using no screen at the time) for exactly fifteen minutes, and then I took the photograph shown in B (× 250) with an exposure of three minutes. The results shown are most interesting. During the fifteen minutes' interval the lowest sphere had evidently moved considerably, though from the sharpness of its outline it is clear that it can scarcely have moved at all during the taking of the photograph. The small upper sphere had evidently moved less, though very perceptibly, during the interval, and had oscillated during the three minutes in which the photograph was being taken, as its outline is so hazy and indistinct. The other small and the large sphere had moved comparatively little.

A rather large sphere of red-orange colour which was a long way out in a filament is shown in C (× 375), after it had been killed by a weak solution of formalin. The sharpness of its outline shows that all movements had been stopped. In some of the large spheres a very rudimentary development takes place. They no longer have the appearance shown in C; they seem to have grown somewhat, since around the heap of pigment granules there is a rim of brownish-yellow protoplasm, such as is to be seen in D (× 250). This body is situated in an empty spore, whose outer membrane shows the rupture produced during germination.

On a single occasion only have I seen one of these pigment spheres encysted. It was situated outside a filament from which it had been liberated.[1] This specimen was in the first stock of these germinating resting-spores that was examined, and was found after the spores had been in my possession, in a small bottle, for five months. The cyst showed a rough tuberculated margin as in E (× 500), and the contents were of a blackish-green colour. Although this body was outside the filament there was no room for doubt as to its nature.

I have now examined two or three hundred germinating resting-spores of Vaucheria, and in every one of them the original pigment heaps have been seen in one or other of the conditions just described—that is, each of them has been found to be included in a small mass of protoplasm which has been formed around it in some way during the stages immediately preceding

[1] The liberation is easily accounted for, as it very commonly happens that after a time the spore itself and one or more of the proximal segments of the filament dies. All the chlorophyll corpuscles of such segments disappear, while the membranes often become soft and disintegrated. The formation of dissepiments in both young and old filaments of Vaucheria is by no means uncommon.

the germination of the spore. So long as the spore has not sent forth any filaments we see more or less ill-defined aggregates of pigment granules—this being the case even up to the stage almost immediately preceding germination, such as is shown in Fig. 21, D and E. On the other hand, as soon as germination has taken place we find these pigment heaps, spherical, sharply defined, enclosed within a scanty amount of protoplasm, and exhibiting slight powers of independent movement, which, as with other low organisms, are destroyed by weak solutions of formalin or osmic acid.

There cannot be a doubt, in fact, that we have to do with the generation, within the resting-spores of Vaucheria, of independent forms of life of a very low order, resembling Amœbæ or the simplest forms of Actinophrys; but forms of life which are so heavily freighted with indigestible matter as to give them but a poor chance of undergoing further development.

Since the preceding account was written I have again examined many specimens taken from the second batch of developed Vaucheria resting-spores contained in the open vessel.[1] These examinations have been made after a long interval, and during this time much of the water had evaporated. Though rather more than ten months had elapsed from the date when the resting-spores were gathered, many of them were found to be still undeveloped. They were mixed with much *débris* from dead filaments, with empty cases of resting-spores, and with a large quantity of pale green Vaucheria filaments emanating from resting-spores which had germinated. Some of these filaments were still in continuity with, though others were separated from, the spores from which they had issued.

Each of the resting-spores in connection with living filaments contained one or more of the pigmented Amœboid spheres. These were now found to be almost motionless, and none of them had wandered out into the green filaments. They were, therefore, probably some of the resting-spores that had recently developed, after long confinement under unfavourable conditions, with the result that the pigment Amœbæ were less active than those which had been produced at an earlier period.

On the other hand, many of the spores and filaments were dead, and from them all, or almost all, the chlorophyll corpuscles had disappeared, though these filaments contained one, two, or more of the spherical Amœbæ—and many of them were in a more developed condition than any I had previously seen. These specimens had lapsed into a resting stage, and were perfectly motionless, but they were seen to possess a wider and more

[1] This addition concerning subsequent changes in the organisms was made on March 20, 1903.

distinct border of protoplasm, stained of a slightly brownish colour but free from pigment granules. Some of these specimens, as in Pl. xiv., fig. 140a, A (× 375), showed clear indications of a commencing segmentation of this peripheral protoplasm; while in others, as in B (× 375), segmentation had actually occurred into a number of minute Monads, whose movements had to be arrested with a dilute osmic acid solution before the photograph could be taken. Two or three of these Monads (rather out of focus) may be seen just escaping, after rupture of the limiting membrane of the sphere.

In other specimens the change that occurred in these more developed pigment Amœbæ, was different. No peripheral, pigment-free protoplasm was developed, but a central nuclear mass of protoplasm, such as may be seen in C and D (× 375), was produced.

All these specimens, as I have said, were found in otherwise empty filaments—that is, in filaments denuded of chlorophyll corpuscles. It is probable, therefore, that they were relatively old, and were relics of some of the earlier germinations.

I have only found a single specimen of the pigment Amœbæ in one of these more developed states, while still contained within an old resting-spore. This specimen is shown in Fig. 140a, E (× 375), and it may be seen to represent a rather abortive attempt at the formation of one of the central nuclear masses.

Both these changes in resting Amœbæ are very familiar to me. They occur frequently in some other kinds of large Amœbæ which are apt to swarm in cells of Nitellæ and in Vaucheria filaments, when these plants have been kept for a short time under certain unnatural conditions. These particular Amœbæ, however, grow most rapidly while gorging themselves with chlorophyll corpuscles. They then pass into a resting condition, and in the course of twenty-four hours, or less, many of them begin to segment peripherally into flagellate Monads; while others, lying side by side with them, and therefore under similar conditions, for some mysterious reason go through the alternative process—that is, each of them gives rise to a single central mass of protoplasm, which becomes surrounded by a membrane, and then remains in a quiescent condition for three or four months. After this long period the substance of the central mass of protoplasm also undergoes simultaneous segmentation into a number of minute flagellate Monads or Zoospores.[1]

It seems clear, therefore, that the sluggish Amœbæ, whose origin has been traced from the mere heaps of pigment granules always present within the resting-spores of certain species of Vaucheria, tend, after comparatively long periods, to pass through

[1] These changes will be found fully described in *Secs.* xix. and xx.

developmental phases of a kind so definite as to remove all doubt as to the fact of their being independent animal organisms—even though they have taken origin from the substance of the plant.

It has seemed best to describe first of all these remarkable changes which appear as part of the ordinary development of Vaucheria resting-spores. They have occurred with such uniformity in hundreds of specimens belonging to two separate batches of Vaucheria, that there can be little doubt about the earlier stages of such processes as I have described in connection with the pigment heaps being always likely to recur in those of the resting-spores that undergo germination. To witness the later stages in the life-history of these pigment Amœbæ they must be kept for long periods under suitable conditions.

I have now, however, to say a few words concerning some other heterogenetic changes that I have seen occurring within those resting-spores of *V. racemosa* that "fail by the way" and never reach the stage of germination.

I have already briefly referred (p. 18) to a heterogenetic change taking place in some of the Vaucheria resting-spores drawn from the bottle containing the first batch of those that germinated. The ultimate result of this change was very similar to what has been seen to be produced in comparatively fresh specimens of the resting-spores of *Vaucheria terrestris* (Figs. 17-20). And now, again, many of the spores taken from the small stoppered bottle containing the second batch which germinated were seen to have undergone similar changes.

The condition shown in Pl. xiv., fig. 141, A (× 250), is very common—in which the resting-spore, in addition to the pigment heaps and fine colorless granules, contains a number of delicate spheres. It is difficult to say whether this is a normal or an abnormal condition, though I rather incline to the latter view, and think it may represent an early stage of such a change as is represented in Fig. 142, E, where the spore was densely packed with Amœboid corpuscles. In other later stages, small separate spheres were often found pretty densely packed within the resting-spores, having each such a very refractive appearance as to make them, at first sight, closely resemble large fat globules—especially when they were in an undifferentiated condition, as were most of those in the small, partly empty spore shown in Fig. 141, C (× 250). Still, on careful examination, a few of the spheres can be seen to contain one of the large central nuclear-like bodies, which subsequently breaks up into microspores. This later stage of the change was, however, seen more frequently in the less refractive, spherical bodies produced within the comparatively fresh resting-spores of *V. terrestris*. One of these spores contain-

ing several of the spheres in an early stage—themselves granular and in the midst of granular matter, partly green and partly decolourised—is shown in D (× 250). In another more enlarged specimen of one of these spores of *V. terrestris*, shown in E (× 375), very similar changes are to be seen; though in other of the spheres (as in the upper of the two specimens to the extreme right), some of them seemed to be breaking up around the periphery into microspores, leaving a small central portion unchanged.[1]

I have seen in one of these resting-spores in the midst of the granular, pale brown residue left, after decolorisation, two rather large, finely granular but very sluggish Amœbæ, represented in Fig. 141, B (× 250); and at other times small Amœboid corpuscles, such as are shown in Pl. xiv., fig. 142, D, E (× 250), either in small or large numbers, within the spores. These bodies represent very common early stages of Amœbæ, such as are to be met with in numerous situations.

Then, again, on another occasion, one of the spores was found containing about eight specimens of Actinophrys, each having very distinct rays, and moving slowly amidst some granular matter. Several of them had red-orange coloured pigment granules in their interior. Another of the spores was found containing as many as 16-20 specimens of Actinophrys, together with granular matter, and a mass of olive-coloured pigment granules. These specimens were not photographed, because I have always found that any solution used to stop their movements causes them to withdraw their rays, and thus renders them quite unrecognisable.

The specimens of Amœbæ and Actinophrys just referred to were seen *within* the thick-walled resting-spores only—*no free specimens were met with outside in association with the spores.*

It thus seems that the substance of the Vaucheria resting-spore changes in two very different ways during decolorisation. Sometimes it occurs more or less after the fashion I have described in the comparatively fresh resting-spores of *V. terrestris* (p. 16), and in the spores of Spirogyra (p. 207); though much more frequently we meet with what I have spoken of as the "common change," in which pigment heaps of varying colour and number are left as residual products. If, therefore, the organisms that appear within these thick-walled resting-spores are somewhat varied in nature, so also are the changes in the constitution of the matrices from which they take their

[1] Such changes are similar to those shown in Fig. 141, A, and will subsequently be found to be extremely common in the large resting Amœbæ, to which I have previously referred as occurring abundantly, under certain conditions, in Vaucheria filaments, and, even more commonly still, in Nitella cells.

origin. Still it must be remembered that Monads, Amœbæ, Actinophrys and Monad Cysts[1] are all most closely related, and more or less interchangeable forms of life.

Such are the heterogenetic products yielded by the varying states of those Vaucheria resting-spores that fail to germinate; while in the spores that actually germinate we have the remarkable pigment Amœbæ produced and going through related changes, as described in the earlier portions of this section.

XVI. ON VARIOUS HETEROGENETIC PROCESSES OCCURRING IN THE RESTING-SPORES OF SPIROGYRA.

The first full account of the actual germination of the resting-spores of Spirogyra was given in 1853 by Pringsheim[2] in an important memoir entitled "*On the Germination of the Resting-Spores, and on a form of Moving Spores, in Spirogyra.*" He says: "While the observations on the conjugation of Spirogyræ, first made by O. F. Müller, have since been frequently repeated and are now universally known, the germination of the spores produced through the conjugation, first seen by Vaucher in 1803, has been confirmed only by very few subsequent observations." Speaking of Vaucher's work Pringsheim says: "He gave a representation of the germinating spores correct in all essential points, but not adequately good and accurate for the demands of our own day. These figures are all that botanical literature possesses." Other observations of the process were made and recorded (though without figures) by Meyer and Alex. Braun.

Pringsheim, however, continues as follows: "Opposed in appearance to these exact observations on the germination of the bodies originating in the conjugated cells of Spirogyra, stands the statement of Agardh that these bodies are broken up into *moving* spores after a certain time; on which account Hassall, who participates in this view, considers these bodies, not as spores, but as the sporangia of the Spirogyræ. Unfortunately, the short account of Agardh, which, although the subject well deserved it, was not accompanied by drawings, does not allow of satisfactory conclusions as to the phenomena observed by him. Meyer had already noticed that secondary—*but not moving*—cells were often formed inside the spores of Spirogyra, and he conjectured that these were likewise propagative cells." Pringsheim also found certain secondary cells within some of the resting-

[1] These are the bodies referred to on pp. 15 and 19; they represent one developmental stage of organisms whose different phases and history will be fully considered in *Sec.* xix., dealing with the heterogenetic changes occurring in Nitella cells.

[2] Translated by A. Henfrey in *Annals of Nat. Hist.*, 2nd ser., vol. xi., pp. 210-224, and 292-300, with two Plates.

spores resulting from conjugation. He adds: "They were always, however, motionless, and I was equally unsuccessful in observing a further development of these cells, and confirming the very natural conjecture of Meyer by direct observation."[1]

I have made the foregoing quotations because they will be found to have an important bearing upon my own observations, and upon the interpretation which is to be placed upon them. Fortunately my observations happen to have been made upon the same species of Spirogyra as those of Pringsheim—namely, *Spirogyra jugalis*, a form having very large spores and filaments.

In regard to the duration of the "resting period" of the spores Pringsheim says: "Conjugated specimens of this Spirogyra, collected in August, maintained themselves in this condition through the winter, in my room, in a little glass vessel full of water, to the bottom of which they gradually sank. Some spores germinated as early as February, but most of them did not open until April, so that some eight months elapsed between their formation and their germination."[2]

I may say at once that the actual process of germination as seen by me has been almost completely in accord with the accurate figures of the process given by Pringsheim, though I have found important changes in the spores, antecedent to germination, to which he makes no reference. I shall principally concern myself here, indeed, with these appearances, and with what Pringsheim speaks of as the "secondary cells" that are apt to form within the resting-spores of Spirogyra, as well as within those of Vaucheria. The bodies in question are, in fact, essentially similar in the resting-spores of both these Algæ.

The specimen of *Spirogyra jugalis* that was submitted to examination by me was found full of resting-spores on July 12, 1902. It was left in an open dish on my work-table for a fortnight, by which time many of the filaments had undergone a considerable amount of decay, though the resting-spores still remained of a fresh and bright green colour.

During this fortnight both the spores and the filaments were examined from time to time. The results of the examination of the filaments will be dealt with in the next section; for the present we are concerned with changes in the spores only.

Some of the resting-spores had evidently been formed for some time, looking to the withered state of the filaments in which

[1] I have not here repeated all the valuable references which will be found in Pringsheim's memoir as to the places where the work of those whom he has quoted has been recorded. Any one interested in this subject must necessarily refer to Pringsheim's important memoir.

[2] In the observations made by Braun the resting period was still longer. He says he saw germination take place "eleven months after the ripe spores had been gathered" ("Rejuvenescence in Nature," Ray Soc., 1853, p. 135, *note*).

they were contained. They were much larger but otherwise very like what are shown in Pl. xiv., fig. 143, A (× 250). These particular specimens were taken from another stock of Spyrogyra. The spiral bands with their amylum bodies were most plainly seen, though they were at this time close together, or even in actual contact. The numerous more recently formed resting-spores presented a very similar appearance, and were contained in fresh, clean looking cells, free from granules or foreign bodies of any kind. All stages of the process of conjugation and the formation of the resting-spores were also numerously represented in this particular stock of the weed.

At the expiration of a fortnight portions of the Alga, with its resting-spores, were put into the same small, loosely covered bottle in which the Vaucheria resting-spores had been kept, and the bottle was left as before on the mantel-piece.

After the lapse of eight weeks the contents of the bottle were first examined. Several portions of the deposit at the bottom of the vessel, removed by a pipette, were carefully investigated, with the result that I discovered only a single resting-spore beginning to germinate, which is shown in Pl. xiv., fig. 143, D (× 125). A few spores were found with dark green bands, no longer in contact as in the early stage of the spore, but showing between them a pale drab granular substance; while others revealed, in these intervals, what appeared to be the early stage in the formation of such spheres as are represented in Fig. 143, B (× 250). The great majority of the spores, however, were either ruptured and empty, or else ruptured and still containing a variable number of Amœboid corpuscles (that is, Amœbæ in a resting stage) mixed with granular matter, as in Fig. 142, B (× 250). Others contained corpuscles of about the same size, enclosing a large fatty-looking central body or nucleus, and having more the appearance of the Monad Cysts which have been found in Vaucheria resting-spores and in Euglenæ (see pp. 18, 19 and Fig. 141, C).

No Moulds of any kind, or free active Amœbæ, were seen in either of the six portions of the sediment taken from this bottle and submitted to the most careful examination.

Several of the resting-spores were found entire and having the whole of their substance resolved into a uniform, granular magma of a pale brown colour, with here and there a tinge of pale green as the sole visible remnants of the bands. This condition is shown in Pl. xiv., fig. 142, A (× 250), and it constitutes, in all probability, the stage antecedent to the appearance of the Amœbæ and the Monad Cysts above referred to. It had resulted from a molecular disintegration of the chlorophyll bands, and their more or less uniform admixture with the central granular matter of the spore. In B (× 250) we have some resting Amœbæ in the midst of very similar matter—others of which in an active

state had doubtless escaped through a crack seen in the wall of the spore.

I have previously indicated that the changes which occur in Vaucheria resting-spores are almost precisely similar to those met with in the Spirogyra spores. And in part illustration of this statement I would call attention to Fig. 142, C, D, E (× 250), in which C shows one of the resting-spores of *Vaucheria racemosa* breaking down into a green and drab granular magma; D shows one of such spores containing only a few resting Amœbæ (others having escaped); while E shows one of the spores quite full of such bodies.

Other portions of the Spirogyra, with its resting-spores, were, two days after it was gathered, placed in a small covered porcelain pot with tap water, so that these particular resting-spores were kept in absolute darkness, and with a very limited supply of air. Some of these spores were also first examined after eight weeks, and they were then nearly all of them found to be in very much the same condition as is shown in Fig. 143, B (× 250), although such spheres as are there shown were not quite so distinct at that time. The green spiral bands were quite obvious but with well-marked intervals between them, which served to show that the granular substance of the spore was densely packed with a number of delicate spheres varying much in size, whose nature was altogether obscure.

Some of the spores were examined again at long intervals, but they seemed to undergo little change, except that the delicate spheres appeared to become rather more definite in outline. In many cases whole rows of spores crowded with such bodies were seen in otherwise empty filament cells—that is, cells completely devoid of Mould, Amœbæ, or organisms of any kind by which the spores could have been infected.

In specimens taken from the pot, just six months after they were put into it, each of the delicate spheres seemed to possess a very minutely tuberculated envelope, so that when these very unequal bodies were focussed half through, their circumference presented a fine milled-edge appearance. The spheres were not at all highly refractive, and many of them had no visible contents, though in others one or more small yellowish-brown granules or nodules were to be seen.

No mention of these peculiar spheres in the resting-spores of *S. jugalis* is made by Pringsheim. Indeed, after the description of the spore in its early state all that he says is (*loc. cit.*, p. 213): "The older the spores grow, the more does the form of the spiral bands in their interior disappear, and their contents become uniformly diffused over the entire inner surface of the spore membrane. Finally, just before the germination, the original spiral arrangement of the contents is still indistinctly indicated

by several close spiral streaks in the coating, spread uniformly over the wall." The account given by Alex. Braun of the changes witnessed by him in the resting-spores of some species (not named) of Spirogyra, also differs notably from what I have seen in the spores which had been confined in the dark pots. He says,[1] " the originally green contents of the spores become sometimes lighter, sometimes darker brown, and appear densely filled with oil drops of different sizes. . . . . The contents of the spore appear totally changed when it is about to germinate; the multitude of large and small oil drops has vanished, and the opaque mucilage, now become green, again exhibits, but indistinctly, a few drops or vesicles."

No oil drops or globules have been seen by me within the resting-spores, and I am inclined to think that Braun must have seen some such specimens in the spores of Spirogyra as I have shown to occur in the spores of Vaucheria (Fig. 141, C). These bodies have much the appearance of large oil globules; and the microscopes in Braun's time may not have been adequate to reveal their real nature.

At this stage of my investigations—that is, after the resting-spores had been in the dark pot rather over six months, and had been in an almost stationary condition for nearly three months—I determined to see what the effect of exposing them to light would be—even in mid-winter, that is, in the month of January. I accordingly took a few of the spores up with a pipette and transferred them to some water in a small tube, which was then left exposed to a rather dim light.

My surprise was great to find on examination at the expiration of three days that every one of the spores had either germinated or was going through the process. And, on three occasions since, the same kind of thing has occurred—that is, the spores taken from the dark pot have, some time during the second or third day of exposure to a very moderate amount of light, begun to germinate.

My examination of these specimens has enabled me to make three interesting observations which I must mention, though they lie quite outside the subject of heterogenesis.

In the first place I found that the delicate spheres shown in Fig. 143, B, during the time immediately preceding germination, open out in a curious sort of way, so as to line the spaces between the re-shaping bands. This I saw while examining the large spore C (× 250). I also found that it was a surface covering of what looked like the most minute air-bubbles which gave the spheres their minutely tuberculated aspect. This is by no means

[1] " Rejuvenescence in Nature," Ray Soc., 1853, p. 202.

plainly shown in the photograph, though it was very distinct in some aspects of the actual specimen.[1] Detail as to this process does not concern us here, but I refer to it merely in order to make known the way in which these peculiar, and so far persistent, spheres (whose nature had been a great puzzle to me) come to an end; and in order to mention my second point, which is that what Pringsheim spoke of as an "entirely new constituent of the spore" appearing "at the time of germination, and in the young plant after germination, and *never absent*," are the yellowish or reddish-brown granules or nodules which I had noted as being present weeks before germination occurred within some, and some only, of these peculiar spheres.

When the spheres open out between the bands these granules and nodules are of course liberated, and the tendency is for them, in the young single- or two-celled plants, to aggregate principally, though not exclusively, around the nucleus. They may be seen in Pl. xiv., fig. 146, A (× 125) to be principally clustered near the centre of this little single-celled plant, and in the midst of them, in the actual specimen, the large but delicate spherical nucleus was most distinctly seen.

The third fact, indeed, that I wished to mention is this, that such a nucleus may be seen in the squeezed-out contents of the resting-spore itself, and that it has been most distinct in all the unicellular plantlets that I have examined, though Pringsheim, speaking of the nucleus, says: [2] "I doubt its actual existence in the spore and in the young unicellular plant"; and he goes on to express his belief "that it originates in the unicellular plant, immediately before the formation of the septum."

Referring now to the quotations from Pringsheim, at the commencement of this section, in regard to views which had been expressed by previous naturalists concerning possible modes of reproduction of Spirogyra, other than by the direct development of the resting-spores, it will be found that there were three different suggestions. They all related to bodies which the naturalists in question believed to have been generated within, and from, the substance of the resting-spores.

The bodies that Meyer and Pringsheim himself regarded as having been thus produced are spoken of by the latter as "secondary cells originating in the spores from their contents." He then makes the following statement (*loc. cit.*, p. 292): "The transformation of the contents of the spores into these cells is by no

[1] This spore rolled about before I got it into a stationary condition and was able to photograph it, and then the best side was unfortunately hidden. The minute air-bubbles are, however, well shown on the surface of the bands in Fig. 146, C (× 375).

[2] *Loc. cit.*, p. 218.

means rare, they present either the appearance shown in fig. 7, of little round cells with granular contents; or, as Meyer[1] represented them, of similar cells, but with contents consisting only of *one single homogeneous* grain, almost entirely filling the cell. I have not been able to detect movement or germination in them." The third suggestion, as to another possible mode of reproduction of Spirogyra from changes taking place in its resting-spores, was that put forward by Agardh,[2] who said, "After many vain attempts to see the elliptical body developed into a new filament, as described by Vaucher, *I saw it, on the contrary, broken up definitively into numerous sporules endowed with a rapid motion.*" With this view of Agardh, Hassall says[3] he concurs, and he accordingly speaks of the resting-spores as "sporangia."

The structures existing in the filament-cells of Spirogyra are, as Pringsheim says, even "more interesting" than those to be found in the resting-spores, so that further discussion concerning the import of the changes in these latter spores had better be deferred till the changes observed by him within the filament cells have also been referred to.

XVII. ON VARIOUS HETEROGENETIC PROCESSES OCCURRING WITHIN THE FILAMENT CELLS OF SPIROGYRA.

The observations now to be recorded were made upon the supply of *S. jugalis* during the fortnight preceding the putting of the resting-spores into the small covered bottle.

Some comparative experiments were made with this weed when cut off from all light rays, visible and invisible, by putting portions of it into a covered earthenware pot; and on the other hand allowing portions of the weed to be simply cut off from ordinary visible light rays by putting them into a dark cupboard in a similar pot, except that the pot was covered by glass instead of by earthenware. The strikingly different results obtained I have referred to on p. 57. That something more was cut off in the first case in addition to ordinary light rays, seems perfectly clear. Some of the invisible light rays must have been excluded by the earthenware cover, which were, nevertheless, able to get through the wooden door of the cupboard and the glass cover. I was wrong in imagining that such invisible rays were Röntgen rays, as it appears that existing knowledge concerning them affords no sufficient warrant for this supposition.

Numerous microscopical examinations of portions of the weed taken from the open dish yielded most interesting results: but, before speaking of them in detail, it will be well to quote a part

[1] *Pflanzen-Physiologie*, iii., pl. 10, fig. 13, *c*, *d*, *e*.
[2] *Ann des Sciences Nat.*, 2nd ser., vi., p. 197.
[3] *Loc. cit.*, p. 130.

of Pringsheim's observations concerning changes of the same, or of a very similar order that were witnessed by him. This will suffice to show how firmly he believed that the organisms seen were formed by transformations of the actual substance of the Spirogyra itself. He says:[1] "I frequently found, namely, in conjugated filaments, that the contents of one or more pairs of conjugated cells were not transformed into the well-known large spore. But while in unconjugated cells in which no spore was produced the contents became decomposed, exhibiting a disappearance of chlorophyll and simultaneous appearance of a red-brown colouring matter, in perfectly indefinite although here and there granular forms (Pl. viii., fig. 1, o), the contents of *such conjugated* filament-cells as produced no solitary spore became transformed into a number of little cells of regular, definite, and unchangeable form (Pl. viii., fig. 4). This regular occurrence led me to conjecture that these cells were more than mere pseudo-forms of decaying cell-contents. I first obtained an insight into these structures by observation of their production in the cells of the young *Spirogyræ*, which I had myself seen emerge from large spores. In the cells of *these young Spirogyræ* the existing spiral bands are often broken up, and from their substance are formed, in a manner still unknown to me, little cells in which a membrane can be clearly detected surrounding green contents (Pl. ix., fig. 8, A)." Pringsheim then goes on to describe the subsequent changes that took place in the contents of these cells formed from the endochrome of the Spirogyra, and leading to the production of "small elliptical cells" which moved about "independently and freely in the filament-cell in the manner of zoospores."

It is the less needful to follow him further in this description because the exact changes occurring in such products are, as I have found, very prone to vary at different times, under slight differences in external conditions, or in accordance with slight modifications in the state of the endochrome itself at the time that it begins to undergo transformation. In the specimens examined by Pringsheim there seems to have been only a comparatively small amount of variation, and this was one of the causes that led him to adopt the notion that the little cells in question were "moving spores of Spirogyra."

In regard to this question of interpretation, that is, as to the nature of these bodies, he speaks as follows:[2] "It appears to me that their *mode of formation* and the *regularity* of their appearance necessarily repel the idea that they are accidental, abnormal productions, without further value in the development of the plant. That they are foreign structures, not belonging to the

[1] *Loc. cit.*, p. 292

[2] *Loc. cit.*, p. 294 The words in italics are as in the original memoir.

Spirogyræ, would be an altogether inadmissible hypothesis, *since they are formed in the interior of the closed filament-cells of the* Spirogyræ *directly from their contents*; for how, supposing them to be Infusoria, should an earlier generation of them have come into a closed cell? Or is it probable that such Infusoria, produced by a *generatio æquivoca*, would begin and end their life in the interior of a vegetable cell?"

This particular improbability in regard to a *generatio æquivoca* is certainly one of Pringsheim's own making, for why should such organisms not be liberated commonly enough into ditches and ponds, as they certainly are, by the softening and rupture of the walls of the cell in which they have been produced? It is a strange difficulty, too, for Pringsheim to have advanced, seeing that in the very next sentence he has to postulate "favourable circumstances" for the setting free of these same bodies, which otherwise certainly never could accomplish the rôle that he would assign to them—that of propagative cells or spores capable of reproducing the parent plant.

Nothing of the kind, however, had ever been actually seen by Pringsheim. He watched them wandering about unceasingly for several hours and then coming to rest within the cell. His words are most definite: "All, however, that I have observed, after they had come to rest, became decomposed without further organic development," and he adds, "I never saw them emerge from the filament-cells in which they had been produced, since no orifice was ever formed in the *everywhere closed* filament-cells."

I will now briefly describe the transformations that I have myself seen occurring within the closed cells of Spirogyræ, and the products of such transformations.

There is good reason to believe that the first organisms that are formed from the transformation of the endochrome of this, as of other Algæ (such as Vaucheria or Nitella), are Amœboid bodies. Spherical aggregates of changing endochrome, differing much in size, gradually go through various changes in colour, and ultimately become completely decolourised, spherical masses of protoplasm.

Such bodies if small may, after a shorter or longer time, gradually begin to change in form, and exhibit the movements of an Amœba or an Actinophrys; or may each throw out one or two flagella and develop into Monads. These, at first, exhibit merely very slow, pendulum-like or semi-rotatory motions, before taking on the more active movements usually characterising such organisms.

Where the Amœboid body, directly produced from the metamorphosis of the endochrome of the Alga, is larger, one or other of two kinds of changes may occur therein, and that before such body has shown any movements of its own.

Its peripheral substance may undergo segmentation into small motionless spheres leaving a central mass of refuse material; or, in other cases, the whole of its substance may become thus resolved. In either case such minute spheres subsequently develop into, and are liberated as, small spherical or ovoid flagellate Monads. It is probable that a process of this sort, occurring within the resting-spores of Spirogyra, is what Agardh and Hassell saw; and that it was a modification of such a process also that was witnessed by Pringsheim in the filament-cells of Spirogyra—giving rise to the little ovoid Monads which he regarded as zoospores. The bodies that he saw, and depicted in his fig. 7, within the resting-spores of *S. jugalis* were, in all probability, bodies exactly similar to those that I have found in the resting-spores of Vaucheria, and which are shown in Pl. xiv., fig. 141, E. Some of the little spheres were simply granular, and others of them (many in Pringsheim's figure) were undergoing segmentation throughout their whole substance.

At other times the Amœboid body becomes more distinctly encysted and forms within itself a large nuclear-like mass of highly refractive, homogeneous and glistening protoplasm. Such a body remains, for a long time, quite unchanged, but ultimately this glistening central substance may break up into a number of minute active Monads, commonly spoken of as zoospores. These bodies, when small, are what I have been terming Monad Cysts; and such are evidently the bodies that were seen within the resting-spores by Meyer — that is, little round cells with contents, as Pringsheim puts it (*loc. cit.*, p. 292), "consisting only of *one single homogeneous grain*, almost entirely filling the cell." Pringsheim himself seems (judging from his fig. 7) to have seen the earlier stage of these bodies—that is, the bodies as they appear before the large central nuclear mass begins to be formed.

In Spirogyra filament cells, as well as in the spores, the foregoing changes are mostly to be seen on a small scale only, that is, in comparatively minute representatives; but in Nitella and in Vaucheria filaments we shall see them occurring in very much larger masses and on a much more extensive scale.

I now proceed to give some illustrations of these processes as I have seen them occurring within the filament-cells of *S. jugalis*, as well as in other smaller forms of Spirogyra.

In Pl. xiv., fig. 144, A (× 125) we have a representation of the failure of the process of conjugation, owing to the contents of the cells in one of the filaments breaking up and aggregating into a number of Amœboid bodies, some of which were observed during examination under the microscope to have undergone peripheral segmentation into minute spheres (though they are not to be seen in the photograph, owing to its low degree of enlarge-

ment). This kind of change was often met with and it invariably occurred in the cells of one of the conjugating filaments only, though Pringsheim does not refer to any such limitation. In B another filament (one of a pair) is shown in a very similar condition, though the little Amœboid bodies are more fully formed in one of these cells.

In Fig. 145, C (× 250) we have in a row of spores a gap, in which, instead of a spore, the cell contains a number of rather large brood-cells, the contents of nearly all of which have undergone segmentation into 10-15 small motionless spheres. These brood-cells or Amœboid bodies seem to be formed in some way during the breaking up of the spiral bands, as Pringsheim has intimated for related products—a view which Arthur Henfrey, the translator of Pringsheim's memoir, confirmed in a note at its close.

Before proceeding further it may be well to point out that a different interpretation has been placed upon these processes by Cienkowski. He describes[1] the penetration of the walls of certain Spirogyra cells by a Monad, which he calls *Monas parasitica.*[2] He says nothing as to whether the filaments of Spirogyra on which his observations were made were very slender with thin walls, or large with thick walls. He does say, however, that "the cell-walls had a much softer appearance than in the normal state—often being themselves almost completely absorbed." It is important to call attention to the fact, therefore, that the observations of Pringsheim as well as my own were made upon one of the specimens of Spirogyra (*S. jugalis*) that has the largest cells and the thickest walls, as well as upon its thick-walled resting-spores—and that no trace of softening of the walls, either of the cells or of the spores, was appreciable before the appearance of organisms in their interior.

Then, again, neither Pringsheim nor myself have been able to observe Monads adhering to and penetrating these thick walls; nor have we seen them moving about within the cells as Monads, then changing into Amœbæ and entering into masses of chlorophyll and cell contents in the obscure manner described by Cienkowski. I have searched in vain for any such appearances, and had they been present my photographs would certainly have shown some evidence of their existence. That Pringsheim was similarly unsuccessful must be obvious to any one who reads his memoir and recognises the strength of his conviction that the organisms seen were formed from the very substance of the Alga.

[1] *Jahrbuch für Wissensch. Bot.*, i., 1858, p. 371.

[2] In *Archiv. für Mikros. Anat.*, i., 1865, p. 213, he speaks of the same organism as *Pseudospora parasitica.*

He did not believe that Monads could either enter or emerge from the thick-walled filament-cells and resting-spores of *S. jugalis*, and definitely says so—after having had them under observation for long periods.

The very conjectural mode of formation of the Amœboid bodies put forward by Cienkowski, and represented by his mere diagram (*loc. cit.*, Pl. xxiv., B, 4, *i*, *á*), is absolutely different from what I have shown in Pl. i., figs. 7 and 11, and Pl. ii., figs. 17 and 18, and which I shall subsequently show as occurring in Vaucheria and Nitella filaments. Otherwise my observations as to the two kinds or states of Amœboid cells that are formed, and as to the products to which they give rise, are in accord with his. There are only a few differences in detail between us. Thus I shall have to show that the whole contents of the thin-walled Amœboid cells may, at times, segment into Monads rather than its peripheral portions only; and that it is not "want of water and other necessities of life" which predispose to the formation of the thick-walled so-called "resting" states, seeing that the two forms often occur side by side within the same cell or filament of Nitella and Vaucheria.

While it may be admitted, therefore, that Monads may be able to penetrate certain thin-walled cells of Spirogyra, such as Cienkowski represents, especially when they are very unnaturally softened and their walls are, as he says "almost completely absorbed," too much stress must not be laid upon this possibility in attempting to explain the observations of others. It has long been thoroughly admitted that green vegetable cells forming integral parts of *Volvox globator* may be gradually transformed into colourless, active Amœbæ[1]; and the observations of Pringsheim and those that I have recorded on *S. jugalis* are only other instances of a fundamentally similar process. The actual form of the organisms produced is of comparatively little moment. The Amœba, the Actinophrys and the Monad are interchangeable forms; and the Amœba after a variable stage of growth and development may (as I have shown for the body formed during the germination of the resting-spore of Vaucheria[2]) either encyst itself and quickly segment, in whole or in part, into Monads; or it may form a thicker cyst, and condense its protoplasm into a more or less central spherical mass which, after remaining quiescent for many weeks, may in its turn also segment into Monads (zoospores). These are, as it were, generic processes in the life-history of small Amœbæ, so that the similarity between the two kinds of cysts (the thin and the thick walled), observed by Pringsheim and Cienkowski, has none of the significance which was attached to it by this latter observer.

[1] See p. 236. [2] See p. 201.

Now comes the question whether any more light can be thrown upon the actual mode of origin of these Amœboid bodies from the substance of the Spirogyra bands.

In some of the Spirogyra cells as shown in Pl. xiv., fig. 145, A (× 250), the so-called "amylum bodies" of the bands become greatly enlarged, and take on exactly the appearance of cells, in many of which what appears to be a distinct nucleus is to be seen. This is a change that occurs within some of the cells of a filament only. Although I cannot positively say that it is the change which precedes the formation of such Amœbæ as are shown in C (× 125) and in Fig. 144, A, B, I have every reason to believe that it is so. In Fig. 145, B (× 250), a further stage of change in the amylum bodies of the bands in this direction is shown. The whole contents of the Spirogyra cell had here shrunk away from its wall, the amylum bodies were still further enlarged and nucleated—and several of them were, as it seemed, being actually converted into Amœbæ.

These amylum bodies, sometimes spoken of merely as "starch granules," are evidently of much more importance than is implied by any such name. They are perfectly distinct in the bands, as first formed, in a single-cell plantlet which has developed from a resting-spore, as may be dimly seen in Fig. 146, A (× 125). Some of the bands and amylum bodies are more highly magnified and therefore much better seen in B (× 375), as they appear in a two-cell embryo; and other amylum bodies, unusually large, are shown in C (× 375) in a single-cell embryo. The bands in this latter specimen are in part covered by the minute bodies like tiny air bubbles, which previously covered the delicate spheres found within the resting-spores. These tiny specks are more numerous on the bands of some embryos than on others. In both B and C the proximal bands of the plantlets are shown; and in C this part of the plant was still contained within the ruptured envelope of the resting-spore.

At these early periods in the life of the plant the so-called amylum bodies, even after soaking for hours in a solution of iodine, do not give the least trace of a starch reaction. Later on in the life of the plant it is different. Wood,[1] speaking of them, says they "are protoplasm, dyed with chlorophyll-green, and are believed to be specially active in the formation of starch. At times iodine turns them simply brown; at others it colours their inner portions blue and their outer brown, showing them to contain starch." Pringsheim,[2] moreover, speaks as follows

[1] *Loc. cit.*, p. 160.

[2] "Researches on Chlorophyll," *Quar. Jour. of Micros. Science*, 1882, p. 82, Pl. viii., fig. 14. The nucleated structure of these bodies is indicated in Figs. 16 and 17.

concerning these bodies: "the threads of the protoplasm extending outwards from the central plasma mass [surrounding the nucleus] in each cell, do not, as was supposed, end in the general protoplasmic lining of the cell wall, but each passes directly, or by its branches, to the internal surface of the chlorophyll band, and there dilates in a trumpet-like manner and grasps, as it were, an amylum body. . . . As the amylum bodies *increase by division*, the grasping protoplasmic thread also divides by forking, and thus each daughter amylum body is grasped by a protoplasmic thread."

Amylum bodies are particularly common and distinct in one or more rows in the chlorophyll layers of Closterium and other Desmids;[1] and so far from being mere "starch granules," as they are sometimes called, they are evidently, in Spirogyra, at least, bodies of some importance in the life of the cells; and capable at times, under abnormal conditions, of undergoing (as we have seen) a remarkable development into indepedent living units of an Amœboid type.

The later stages of such Amœbæ as have taken origin from the developed and individualised amylum bodies are shown in Pl. xiv., fig. 147 (× 250). In A the peripheral contents of the encysted bodies may be seen to have undergone segmentation into embryo Monads, leaving a large amount of unconverted residual matter in the centre of the cells. In B a small and also an unusually large encysted Amœba seems to have undergone complete segmentation into embryo Monads, some of which have evidently become active and escaped from each of the cysts. These will be found to be exactly the kind of products represented by Pringsheim[2] as appearing within the developed zoospores of a species of Œdogonium.

On other occasions the breaking up of the chlorophyll bands in cells where the process of conjugation fails, seems to yield Fungi rather than Amœbæ. I have seen many cells in such a condition where not a trace of Mould could be found on the outer side or around the filaments. Sometimes the two kinds of product (Amœbæ and Mould) co-exist within the same cell, as was the case in C (× 250) and in D (× 125). In the latter specimen the Spirogyra cells on each side seemed perfectly healthy. It may be thought that this latter fact points to the probability of infection from without in the case of the cell whose contents have become transformed.

This kind of isolated change is, however, common enough in cells which do not yield Mould, but only Amœbæ or Monads (as in Pl. xiv., fig. 148, D) (× 200), and their entry into the closed and

[1] See Cook's "British Desmids," Pls. viii.-xiv.
[2] *Loc. cit.*, Pl. ix., fig. 12.

thick-walled cell of the Alga would be far more unlikely, and is, moreover, a kind of possibility which has already been considered, and shown to be improbable, except occasionally in thin-walled cells, and where the walls have become altogether unnaturally softened.

Changes of various kinds in single cells of Spirogyra, occurring side by side with healthy cells in the same filament, are indeed common enough. The explanation too is perfectly simple seeing that each of the cells is, as Wood says, "apparently independent of its associates." He adds (*loc. cit.*, p. 160): "Each cell in one sense is, therefore, a perfect, complete individual, capable of living dissociated from its companions. How far the life of one of these cells is influenced by that of its neighbours is uncertain, probably to a slight extent, possibly not at all. At any rate, they are so far independent that the filament is rather a composite body than a unit of life." The researches of Pringsheim on Chlorophyll [1] have moreover shown that hypochlorin, an invisible constituent thereof which "plays an important part in the nutritive function of green cells," exists to a very variable extent in such cells even of the same Alga, and that it may be entirely absent from one or more cells in a tissue otherwise rich in this substance. In this and in other ways, therefore, cells apparently similar in appearance may be really very different in constitution and prone to undergo changes not shared by their neighbours. Pringsheim says, indeed, a few pages further on, "It is possible that some of the cases already referred to where one or more cells in the midst of a tissue rich in hypochlorin show no trace of this substance, and in which the hypochlorin was supposed to have been completely used up in the nutritive processes, are instances of disease. The cells, though not visibly so, may be really in an abnormal condition, which has resulted in, and is made known by, the loss of their hypochlorin."

Such heterogenetic transformations as I have been describing in *S. jugalis* I had long previously found occurring in other smaller species of Spirogyra. Only in them the stages in the production of the Amœbæ, of specimens of Actinophrys, or of Monads, which were found within the cells, were not so definitely traced. Some few examples, however, may be referred to.

In a species with short cells and thick bands the contents of some of the cells shrank away from the walls and underwent certain changes resulting in the production of five or six spherical Amœboid bodies, such as may be seen in Pl. xiv., fig. 148, A (× 250). Such masses gradually become decolourised and converted into delicate spheres of protoplasm, still containing some

[1] *Quar. Journ. of Micros. Science*, 1882, p. 81.

residual green or reddish-brown unassimilated pigmentary matter (as in C, × 250). These delicate spheres may develop into active Amœbæ or into Monads. At other times the contents of Spirogyra cells have been seen to separate into spherical masses, which, while becoming decolourised, project pseudopodia and take on the forms of Actinophrys, as they did within the great Conferva cells shown in Pl. i., figs. 8, 9, 10. Another kind of Spirogyra with longer cells is shown in D (× 200), in which the terminal cell of a filament is seen containing a number of Amœboid bodies only partially decolourised. The early stage of similar bodies may be seen in B (× 250), which is a germinating zoospore of an Œdogonium, the bodies contained therein being closely related to those described and represented by Pringsheim (*loc. cit.*, Pl. ix., fig 12). The smaller bodies when decolourised develop into Monads, while the larger probably remain as encysted Amœbæ and undergo one or other of the changes already described.

After what has now been said the view that the flagellate moving organisms, observed by Pringsheim in the filament cells, or the motionless spheres found within the resting-spores of Spirogyra, have anything to do with the reproduction of that plant must be regarded as entirely devoid of foundation. Cooke[1] similarly dismisses the view of Agardh and Hassell as to the resolution of the resting-spores into propagative zoospores. He says: "It need scarcely be added that this view is erroneous, the resulting body [the spore formed by conjugation] germinating direct after a period of rest."

All the observers that I have quoted, however, Agardh, Hassell, Meyer, Pringsheim and Henfrey, were perfectly satisfied that the various bodies seen by them, either within the resting-spores or within the filament-cells, were formed directly by a process of transformation from the actual substance of the Spirogyra. If, therefore, the organisms thus originating had nothing to do with the reproduction of Spirogyra, they were clearly heterogenetic products. There is no room for any other interpretation.

The testimony of another excellent observer may, however, be added. Alexander Braun in his important work "Rejuvenescence in Nature"[2] speaking of what Nägeli termed "abnormal cell formations," and of his own observations in the same direction says: "The character of these abnormal cells is most varied and changeable. . . . Abnormal structures of this kind have doubtless often been confounded with the normal reproductive

[1] "British Fresh Water Algæ," vol. i., p. 76.
[2] Translation in Ray Soc., "Botanical Memoirs," 1853, p. 281.

cells of the Algæ. The future will certainly unfold many interesting phenomena in this hitherto little-worked field."

XVIII. ON VARIOUS ADDITIONAL HETEROGENETIC PROCESSES OCCURRING IN EUGLENÆ.

I have already described and illustrated a number of heterogenetic changes met with in these extremely variable organisms, Euglenæ.

On p. 11 the origin of one or more Amœbæ from their substance was described; on p. 13 the origin of flagellate Monads and also of Peranemata; on p. 19 the origin of Monad Cysts; on p. 110 their transformation into Ciliated Infusoria; on p. 188 their transformation and growth into Vaucheria filaments; on p. 190 the individualisation and liberation of their chlorophyll corpuscles with their growth into a Microspora; and on p. 191 a very similar change in other smaller specimens with growth of their corpuscles into a minute Conferva. This list, however, amazing as it may seem, represents less than half of the total heterogenetic changes met with in these organisms.

Further, I have on p. 181 traced the origin of some Euglenæ from the fission products of a small Algal epiphyte growing on, and within the sub-stomatal spaces of, the two common Duckweeds.

Euglenæ are well known to multiply freely by fission, but, apart from this, no other mode of increase is recognised or has, I believe, ever been described in works on Natural History. No sexual reproduction and no true germs or spores of any kind are known to be concerned with their increase in such prodigious numbers in many sites.

The forms of Euglenæ, and the condition of their internal substance (both in their free and encysted states) are found to vary immensely. These changes in state and in molecular composition in specimens obtained from different localities, where the constitution and conditions of the medium in which they live vary much, may be considered to account, however imperfectly, for the very varied nature of the transformations which they are prone to undergo. The variations in the medium which they inhabit must be extreme, seeing that they are found at times on the surface of the brown fluid draining away from manure heaps in or near a farm yard; while at other times they may be found on the surface of the clear waters of some pond, or left as a scum on the surface of merely damp mud. Then again, apart from variations of temperature, the degree in which these organisms are exposed to direct sunlight is another powerful cause of variation—though one which is probably not so potential for the causation of heterogenetic transformations as the mere bringing

them into the comparatively confined air of a house. This of itself seems to be a potent cause of change for these sensitive organisms, even apart from keeping the vessel in which they are contained under a bell-jar, or putting it into a dark cupboard, or keeping some of the Euglenæ for days in a closed earthenware pot, where they are exposed not only to a very limited air-space, but are cut off altogether from the influence of visible and invisible rays of light.

It must be borne in mind, therefore, that the changes in Euglenæ which I have described, as well as those which I am about to describe, have been found in organisms varying much among themselves and submitted to one or more of these unnatural or artificial variations in their environment.

Some few of the variations in form and state of these protean organisms are represented in Pl. xiv., fig. 149 (all × 250, except B) in which A represents some of the more common forms, and B (× 150) unusually elongated types, the body substance of both being green with a red "eye-speck." In C an organism in an encysted state is shown which was of a bright red colour except for a peripheral arrangement of green chlorophyll corpuscles; the cyst is here delicate and so hyaline that it is scarcely visible. In D we have specimens of a rather large green Euglena, in great part composed of a number of decolourised globules, taken from the surface of a small lake where they formed a pale emerald-green scum; while in E we have two other encysted specimens (one at rest and one slowly revolving) in which the body substance is packed with larger, more defined, and more uniformly decolourised corpuscles. In F an unusual mode of multiplication of Euglenæ is shown, taking place within comparatively thick, slightly yellowish cysts; the result being the production of four or more organisms, such as may be seen emerging from one of the ruptured cysts. As a rule Euglenæ multiply by a simple process of fission into two while within a thin, recently-formed cyst; they never undergo self-division while in the unencysted, active state.

This figure only gives some slight indication of the many differences to be met with in the form and constitution of the common *Euglena viridis*. There is, for instance, an elongated type somewhat resembling in form the organisms shown in B, whose representatives have only very sluggish movements and whose envelopes are covered with a double set of spiral markings. On the other hand Fig. 156 shows a form whose envelope becoming rather brittle as age advanced (judging from the way in which it splits under slight pressure) is covered with very pronounced spiral markings in one direction only.

It is not to be supposed that anyone who merely reads my account of the various transformations occurring in these organ-

isms, even though backed by the photographs, would be so easily convinced as to their reality as if he had actually worked at the subject for himself, and had had the opportunity of seeing very many repetitions of this or that particular change occur side by side, where previously there had been only a uniform and even stratum of Euglenæ.

(*a*) The Conversion of the entire substance of small Encysted Euglenæ into specimens of Actinophrys.

The changes now to be described occurred in a stock of small Euglenæ which had been kept for nearly three weeks in the month of October outside a window, but in the shade and under a bell-jar. When examined after this interval the Euglenæ, gathered on the surface and forming a pellicle, were found in an encysted condition. Many of them had gradually become converted into a mass of reddish-brown corpuscles, and in some no definite eye-speck was visible. Other of these organisms were almost decolourised, though packed with the same kind of refractive and fatty-looking corpuscles.

I at first thought that most of these latter Euglenæ were dead, but on further examination I found among them what appeared to be a mere aggregate of similar fatty-looking corpuscles with a number of fine rays projecting therefrom. I soon discovered that this body was not really stationary as it at first seemed, but that it was moving very slowly, and was clearly an Actinophrys. Others were speedily found of like kind, but two or three attempts to photograph them proved failures, by reason of their movements. Killing them with formalin or osmic acid solutions did not prove helpful, as these re-agents invariably led to retraction of rays and the conversion of the organism into a shapeless mass, if it did not produce actual dissolution.

Further careful examination of portions of this pellicle showed appearances clearly indicating the origin of these specimens of Actinophrys, which looked at first like mere aggregates of fatty-looking globules. The contents of the Euglena cysts, packed with similar globules, which I at first thought to be dead, were, in many cases, traced in different stages, making their way out through their softened cysts, and when outside they were seen to have projected rays, and to be moving almost imperceptibly after the fashion of Actinophrys. Though I saw many dozens of specimens in different stages of their exit from the cysts, they always at such times seemed perfectly motionless, and were in fact so stationary that I was in several cases able to photograph them successfully without making use of any lethal fluid.

A portion of this Euglena pellicle is shown in Pl. xiv., fig. 150,

A (× 250), and it will be seen that all the organisms are encysted; that some of them have undergone fission within the cysts; that some are motionless and others (as indicated by their homogeneous appearance) are slowly revolving; while above and to the left of the centre one body is seen to be emerging from a cyst whose walls are somewhat dilated and probably softened.

The mode in which exit from the thus altered cyst is effected is as follows. A projection from the body substance of the transformed Euglena is pushed against some part of the softened cyst wall, and a few of its corpuscles included within an almost invisible sheath of protoplasm becomes projected through it, as shown in one of the bodies represented in B (× 375). This perforation of the cyst once achieved, the process is soon carried still further in the fashion well shown in C (× 375), where the pointing of the body and the bulging of the cyst wall in the direction of exit is well seen. In D (× 375) a further stage is represented, in which the Actinophrys has half emerged from the cyst. I am right in speaking of it as an Actinophrys, even at this stage, for, in the specimen shown in B, I distinctly saw three rays projecting from the posterior rounded part of the organism, although they were too delicate for representation in the photograph.

In E (× 375) one of these specimens of Actinophrys which had numerous well marked rays is shown after the application of an osmic acid solution—now with all its rays retracted. This specimen showed no eye-speck, but in another, otherwise similar, the eye-speck had been left. In F (× 375) the remains of another are shown after complete dissolution had been brought about by the use of a formalin solution. Thus photographing the organism as an Actinophrys was found to be impossible.

There was no possibility of error about these observations. They are not only interesting as being the first occasion on which I have seen the entire substance of a Euglena transformed into an Actinophrys, but also on account of the additional facts that I am now about to record.

(*b*) The Development of a kind of Polyphagus within the Actinophrys derived from the Transformation of a Euglena.

Numbers of the specimens of Actinophrys within the cysts of which I have just been speaking showed a small specimen of Polyphagus developing in, and projecting from, their substance. One of the earliest stages is to be seen in the lower of the two specimens shown in Pl. xiv., fig. 151, A, in the form of a small rounded projection. This projection in B (× 375) is seen to be larger, while in C the body has grown out into an elongated sporangium with granular contents—smaller, though otherwise

very similar to that of the common *Polyphagus Euglenæ.* At a later stage this sporangium would doubtless break up into a multitude of very minute swarm spores, though not a single specimen in this stage was met with; nor was a single one of the minute nucleated swarm spores seen in any of the numerous specimens of this pellicle that were submitted to examination. Most of the specimens found were in one or other of the early stages such as I have represented in A and B, and only very few were seen with the sporangium elongated as in C.

These sporangia were, moreover, found only in the specimens of Actinophrys which had been derived from the transformation of Euglenæ; none were ever seen in the Euglenæ prior to this transformation. This curious association, though different in many respects, suffices to recall what I have recorded in the first case referred to in a following sub-section (*e*).

(*c*) The Origin of a new kind of Polyphagus from the substance of Euglenæ.

After Euglenæ have been kept indoors for a few days, and especially if they have been kept in a dark cupboard and exposed to a small amount of air by covering the vessel in which they are contained with a small bell-jar, Polyphagi begin to make their appearance. Sometimes several days before the common form (*P. Euglenæ*) appears and spreads destruction around it by means of its infecting branchlets,[1] I have seen a smaller variety begin to manifest its presence here and there, sporadically, among healthy Euglenæ in the midst of which, in previous examinations, no traces of any such organisms were to be found.

The substance of the Euglenæ in which this Polyphagus is developing becomes broken up and discoloured of a red-brown tint, and in its midst some lobulated, pellucid-looking growth is to be seen. Later, this pellucid growth begins to project as in Pl. xiv., fig. 152, A (× 375); and goes on increasing in size so as to form irregularly-shaped sporangia such as may be seen in two of the specimens represented. Very early in their development these sporangia show nuclear bodies here and there, as indications of an approaching segmentation into minute zoospores, such as may be seen more highly magnified emerging from a partially crushed sporangium in C (500). These zoospores are spherical, rather than of a rounded oval shape as in *P. Euglenæ,* they are also much more minute, and furnished with a proportionately smaller nucleus. The sporangium is also connected with the growth within the Euglena in a curious way, by means of a bent dilated tube such as is shown in B (× 375), in a

[1] A representation of which may be found in De Barry's "Fungi, Mycetozoa, and Bacteria," Translation, 1887, p. 162, fig. 75.

specimen that was fortunately compressed in just such a manner as to make it plain. This dilated tube is usually in part contained within the Euglena, but it generally shows as a small rounded projection at the base of the sporangium, as may be seen in each of the two specimens represented in A. I have never observed the swarm-spores of this form germinate, and have seen no evidence to show that they ever form branching threads, such as are produced from the spores of *P. Euglenæ*. Certainly the Euglenæ that are affected in this manner generally lie apart from, and are in no way connected with, one another. When the Euglenæ in their fresh state are examined no trace of any such change is to be met with, but it commonly enough occurs in the course of a few days, when they have been kept under the conditions that I have mentioned.

This is a change that occurs in non-encysted Euglenæ, but the specimens so affected are from a very early stage rendered quite motionless, and are soon destroyed.

(*d*) The Origin of two forms of Olpidieæ within Euglenæ.

The first of the changes that I am now about to describe is one that occurs with great frequency in many Euglenæ after they have been kept indoors in open vessels for a week or two, but with much more frequency and certainty among Euglenæ that have been shut up within a small covered earthenware pot for a few days.

Different stages of the process are represented in Pl. xv., fig. 153 (× 375). In A some of the early stages are seen, and also one specimen in a late stage. The earliest stage is shown by the appearance of a rather ill-defined whitish spot or patch in the posterior part of the body of a still active Euglena, otherwise unaltered in appearance. This patch is not generally far from, and when seen under a low power is not unlike, a rather enlarged nucleus. But on closer examination with a high power (of about 600 diameters) the nucleus will be found to be full of delicate, evenly disposed granules, while in its earliest stage the new body presents an almost homogeneous appearance, and a motionless vacuole may often be seen near its centre. It has also a definite spherical outline, though this is often obscured by overlapping chlorophyll grains. Later, very minute gemmules may be distinguished in these bodies (rather larger than the mere granules of the nucleus) such as were seen in the upper two bodies of A, and are to be seen in the photograph, though not in its reproduction. As this body increases in size so do the gemmules up to a certain point; after that they only increase in number, as in B, C, and D. As soon as the gemmules become fully formed, the Euglena itself in other parts loses its green

colour, and in the process gives rise to small aggregates of refuse pigmentary material of a red-brown colour, often situated near or around the red eye-speck which persists for some time longer.

Towards the close of these changes the whole body of the Euglena may be almost filled with these motionless gemmules, as in C; though at other times, and especially in earlier stages, they are certainly enclosed within a delicate limiting membrane as in D. In the later stages the Euglenæ thus altered tend to become spherical and motionless, though I have seen specimens, exactly like C, still creeping about in a very slow fashion.

For the most part these changes occur in free and active Euglenæ; it has only been on rare occasions that I have seen such a change within encysted specimens as in D. And even in these latter cases the change may of course have commenced before the Euglenæ became encysted.

It has only been very rarely also that I have seen a swarming movement of the gemmules within the body of the Euglena. Their subsequent fate I have not been able to ascertain as, though I have examined hundreds of Euglenæ in this condition, I have never seen the gemmules discharged therefrom. Looking at all the circumstances, therefore, infection seems highly improbable. It may be regarded as certain, however, that these gemmules have nothing to do with the normal life history of Euglenæ.

On one occasion many small Euglenæ which had been shut up in a small dark pot for over a fortnight had undergone this change, and so completely that they were quite filled with the gemmules. They had got rid of all or nearly all the refuse matter,[1] and having projected short rays from their small amount of peripheral protoplasm, they were moving slowly like specimens of Actinophrys.

The second of the two changes to which I am now referring was seen in some rather large, red Euglenæ, obtained from a pond at Totteridge. They were shaken up during a walk and journey by train occupying about two hours, and on reaching home were put, with some fresh water, into a shallow dish which was left on my work-table. Although they were exposed to actual sunlight during the next two days, for four or five hours in all, very few of them rose to the surface—they remained almost motionless at the bottom of the dish. This I have found liable to happen when Euglenæ have been brought from a distance in water, and consequently have been for a long time shaken up in a bottle. It is better to bring them home in a box, on wet blotting paper or a wet dock-leaf; or if they are found lying on mud some of the surface of this may be scraped off with a large section-lifter

[1] Probably by ejecting it, as I have often seen done by ciliated Infusoria.

and placed in a box lined with two or three sheets of paper. On reaching home this mud coated with Euglenæ is placed at the bottom of a suitable vessel, and water is added thereto very slowly and gently. The mud has become inspissated owing to absorption of its water by the paper, so that water may now be added to the vessel without producing much turbidity, and, when its depth is not too great, if the vessel has been placed in the open air and exposed to a little sunlight, in the course of one to two days a good Euglena pellicle will have formed on the surface.

It was when examining some of the red Euglenæ from the bottom of the dish on the third day after they had been brought home, that I first noticed the bodies now to be described, and I continued to find large numbers of them during the next week in Euglenæ which were not encysted but which, even during the early stages of the change, exhibited only very sluggish movements and later on were quite motionless.

The first noticeable change was of the kind represented in Pl. xv., fig. 154, A (× 250), in which the Euglena shows in the midst of its substance an enlarged nucleus (more plainly visible than is usual in Euglenæ) and also another more or less spherical colourless body, which in the specimen represented happens to be rather larger than the nucleus. Other specimens will be met with in which the new body may be found larger and larger still, so that after a time it generally hides the nucleus completely. As this body increases in size it is seen to have assumed a distinctly oval shape, to be neatly defined, finely granular and often to show a central nucleus or vacuole, as in B (× 375). Later, the substance of the Euglena seems to become soft, much altered, and of a brownish-red tint. The weight of a cover glass often presses the great ovoid bodies out and away from the degenerated Euglena, and when seen free in this later stage a small rounded projection is recognisable at either extremity, as in C (× 375). Later still, the granular mass undergoes simultaneous segmentation into a number of minute motionless swarm-spores such as are to be seen in D (× 375)—where the sporangium lies by the side of the faded, red-brown mass of the Euglena from which it has been expressed.

These swarm-spores, doubtless, after a time become active, and make their way out from the sporangium by the giving way of the nipple-like projections at each extremity. I have never, however, seen them make their exit, or even moving within the sporangia, though I have examined a large number of specimens.

One of the largest Euglenæ that I have ever seen was found among this stock. It was also red, and contained within its substance a very large sporangium of the kind just described, in a well-developed condition, as shown in E (× 375). Around it, above and to the left more especially, its blood red substance (not

faded) was seen more or less compressed, while above, to the right and below, were a number of large and small Amœbæ. Several of the larger specimens were seen to be nucleated and to contain more or less of the red pigment. They were almost motionless, as may be seen from the photograph, which was taken with an exposure of five and a half minutes. The examination was made in the month of July when the temperature of the air was 78° F. The specimen, after being photographed, was left in a Petri dish, still under the cover glass, for eight hours, when another photograph was taken with a similar exposure. It showed that a good deal of movement of the Amœbæ had taken place both before and during the taking of this second photograph.

These sporangia were found within organisms that were not encysted, so that it may be supposed that the Euglenæ had become infected by one of the motile swarm-spores effecting an entry. This, of course, is possible, but then we must not lose sight of the fact that no motile zoospores were seen with the Euglenæ. This is a fact of some, but not of much, value. Evidence against infection of a more cogent character, both here and in the previous case, exists in the fact that never more than a single sporangium was ever found within either of the Euglenæ affected in these ways; and a similar fact was noticed on another occasion when I met with a large number of ordinary green Euglenæ, each containing a single sporangium of the kind above described. If infection had occurred, and a swarm-spore had in some way grown into the great sporangium, why should only one be found in each Euglena? It might surely be expected that oftentimes there would have been a multiple infection, leading to the growth of two, three, or more sporangia within a single Euglena.

(c) The Origin of two other forms of Olpidieæ within Euglenæ.

On p. 11 I have described the production of Amœbæ from a particular stock of Euglenæ; and on p. 13 the production of Peranemata from other specimens of the same stock, obtained from the surface of a small lake near Loughton.

The changes were first recognised after the Euglenæ had been in my possession for about ten days. From 6-10 embryo Amœbæ or Peranemata were produced within each of the Euglenæ that underwent this change, and strange to say in many of each kind there was found with these organisms one, or sometimes two, pale brown, tuberculated spheres, rather larger than the Amœbæ or the Peranemata. These spheres generally showed a nucleus or vacuole in their interior, and as the other bodies grew and the Euglena cyst became softened, the pale brown sphere became wholly or partially extruded.

In Pl. xv., fig. 155, A ($\times$ 80) a few of these Euglenæ filled with Amœbæ or Peranemata are shown under a low magnification; the contained organisms appearing as small whitish spheres. In B ($\times$ 375) two of the pale brown tuberculated bodies are to be seen within one of the Euglenæ. In C, D ($\times$ 375) one of them is seen partially extruded; and in E ($\times$ 250) two of these brown bodies are seen, isolated and under a lower magnification, which were found after an interval of about ten days. They had by this time become rather more tuberculated on the surface, the vacuole had disappeared, and from the larger of them (now empty) a short, wide exit tube was seen. This kind of tube has been several times noticed, and though I have never been able to make out any segmentation of contents, I presume that division into microspores must occur, and that these are ultimately discharged through the short tube above referred to.

I imagine that these brown bodies must be primitive Sporangia pertaining to some representative of the Chytridiæ—probably another of the Olpidieæ, some of the sporangia of which have, according to de Barry, a delicately spiky envelope.[1] I have never been able to make out the mode in which they originate within the Euglenæ, though I have traced the mode of origin of the Amœbæ and Peranemata with which they are associated. Still, like the latter, the sporangia have always been seen only when of full size, and never growing up through smaller grades, as must have been the case if these particular Euglenæ, whose substance was being transformed into Amœbæ or Peranemata, had been infected by the microspores of one of the Olpidieæ.

Such sporangia have never been seen before or since within Euglenæ; and in those that contained them there was for the most part one only, and never more than two. They were never found alone, but only in those Euglenæ whose substance was being transformed either into Amœbæ or into Peranemata.

The specimens next to be referred to were found in some small green Euglenæ, obtained in the month of November, which were subsequently left with water in a photograph dish, outside a shady window but under a bell-jar. At the expiration of fourteen days a number of the Euglenæ were found forming a deposit at the bottom of the dish. They were in a quiescent state though not encysted, had a remarkably uniform and symmetrical ovoid shape, and were mostly of a dark green colour.

I soon found, however, that many of them were decolourised, and in these specimens I was able to recognise more plainly that their envelope was firm, and showed strong spiral markings, such as are represented in Pl. xv., fig. 156, A ($\times$ 375). The surface of

[1] *Loc. cit.*, p. 167.

this organism is focussed, so that its contents are only very indistinctly shown and need not be referred to. In B, within another similar envelope, a rather coarsely granular colourless mass is to be seen, having a definite limiting membrane but showing no nucleus. Another of these bodies is represented in C, in which the contents were much more finely granular, and where there was a large central vacuole or nucleus. Larger masses of pigment granules are also to be seen around the sporangium. In D one of these Euglenæ is shown in which a smaller sporangium is represented by an empty envelope only—its contents having probably been voided in the form of swarm-spores. I have seen several similar empty envelopes, but have never witnessed the segmentation of the contents of the sporangium—so that in all probability the process takes place rapidly and the spores become motile at once, unlike those formed within the two kinds of sporangia described in the last sub-section. On only two occasions have I ever found two sporangia within a single cyst, One of these specimens is shown in E. It had been found alone, and among a different stock of Euglenæ, many months previously. The Euglena is evidently of the same kind, but its contents have more the appearance of resting Amœbæ; and they were at the time entered in my note-book as such. In one of these bodies a nucleus is distinctly visible.

I searched diligently for several days in my comparatively small stock of these Euglenæ, but was unable to make out anything quite definite as to the mode of formation of the sporangia. I found absolutely no indication in the green Euglenæ either of an enlarged nucleus or of a small sporangium gradually increasing in size as in the case of the Euglenæ described in the last subsection. I found, however, a few specimens such as I have shown in F (× 375) in which the contents, still green, had undergone some amount of contraction and had shrunk away from the outer envelope; and two others in which further contraction and decolourisation had occurred. Thus in G the contents had shrunk into an ovoid mass, for the most part decolourised, but with some parts still presenting a greenish hue; while in H the mass was almost completely decolourised, was still smaller and surrounded by some amount of reddish brown refuse matter. I can throw no further light upon the origin of these sporangia.

There is the same difficulty as before in accounting for their presence as a result of infection—an even greater difficulty, because no evidence of gradually increasing size was ever met with, and because of the thickness and hardness of the spirally-marked envelope of these Euglenæ. The difficulty common to this case, as well as to the two forms described in the last subsection, is the fact that, with the two exceptions mentioned, only a single sporangium has been found in each Euglena. If micro-

spores (never seen) had been swarming around them, what was to prevent a Euglena being attacked by several of them simultaneously or successively, and how are we to account for the attack upon the doomed Euglena being left to a single microspore?

It seems pretty clear that these bodies, as well as those described in the last sub-section, belong to that division of the Chytridieæ known as *Olpidieæ*, concerning which de Barry says (*loc. cit.*, p. 167), "this group of species is imperfectly known and needs further investigation." A similar remark may indeed be made concerning other groups of this family, such as Rhizidieæ (to which Polyphagus belongs), Synchitrieæ and Cladochytrieæ. One set of this latter group is spoken of by de Barry as "intracellular parasites in the living and otherwise sound foliage of some of the marsh plants." But he says the "germination of the swarm-spores has not yet been observed . . . swarm-spores of *Cladochytrium Iridis* will not germinate even on dead tissue of Iris pseudacorus. They seem to require living cells for their further development, but *I could not see that they made their way into them.*" And speaking of another genus, Physoderma, whose representatives are found in different phanerogams "*in the inner layers of the parenchyma of the leaves,*" de Barry also says (p. 166), "We are not told how their primordia find their way into the interior of the cells." It is perhaps now well to entertain the question, and seek to determine whether these primordia do not actually originate within the cells of the "inner layers of the parenchyma," rather than effect an entry from without under what would appear to be insuperable difficulties?

(*f*) The origin of another representative of the Chytridieæ within Euglenæ.

The change now to be described took place under the following conditions. Some Euglenæ eleven days after they had been procured, in the month of January, and had formed a pellicle in a bowl kept outside a window, were transferred to a small glass vessel containing tap water. This vessel was placed in a cupboard and covered by a small inverted beaker. On examination of portions of this pellicle four days later some of the Euglenæ were found to contain the small new Polyphagus already described, while just as many of them were found to be altered in the following manner.

The Euglenæ undergoing this change were found to be more or less markedly bulged in a very irregular manner, owing to the presence within them of a translucent lobulated growth in the segments of which a few fine refractive granules were to be seen.

The remaining portions of the substance of the Euglenæ were at first of a pale green, and later, of a pale olive tint. They were never of the reddish-brown colour which I have described as so commonly presenting itself in other changes.

A well-advanced stage of this condition is shown in Pl. xv., fig. 157, A (× 375) in the specimen on the left, while its companion shows a rather later stage, in which the Euglena has become more prominently bulged, and in which two tubes (seen below and to the right, but out of focus) have been sent out from the loculated growth, and through which minute swarm-spores are discharged. Usually only four or five of these tubes of exit have been seen, but in B a specimen is shown from which they were very numerous. After the discharge of the swarm-spores the remains of the Euglena shrink considerably, and only the walls of the loculi are to be made out, together with a few refuse reddish-brown granules. Two of these shrunken bodies are shown in C (× 375).

Sometimes, however, the growth passes through a different phase. Instead of going on at once to the formation and discharge of swarm-spores, the loculi seem to become converted into a number of resting sporangia, and then to remain unaltered for a considerable time. An early stage of this change is shown in D, and a later stage, where the separate sporangia have well-defined limiting membranes, in E. The subsequent changes in these sporangia I was unable to ascertain.

This is a very rare change. I have never met with it on any other occasion, though that described on pp. 18 and 19 seems to be very closely related thereto—the bodies then found being very similar to what I have spoken of above as resting sporangia.

There can be little doubt that all the bodies described in these last five sub-sections are representatives of one or other group of the *Chytridieæ*, and that the phenomena recorded would by most persons be regarded as so many results of infection by means of swarm-spores. Fortunately the mode in which the swarm-spores of one of the representatives of this family infect their host has been carefully studied and made out by Cienkowski. We have, therefore, some definite observations as to the mode in which infection is brought about, the comparison of which with the observations I have just been recording may help others to judge whether the interpretation I have put upon my observations is or is not well founded and justifiable, even independently of the light shed by so many other observations recorded in this volume.

In the communication referred to[1] Cienkowski has described

[1] *Botan. Zeitung*, April 3, 1857, p. 233, Taf. v., figs. 1-6.

and illustrated the mode in which a species of Rhizidium infects the filaments of *Conferva glomerata*. His figures show numbers of the swarm-spores attached to the walls of the filaments, and the different stages by which they effect penetration thereof. The process for any one swarm-spore occupies, as he says, between two and three hours, and as many were seen simultaneously attached to a filament, and in different stages of penetration, such bodies must have been visible for many hours on, and in connection with, the wall of the filament which was being infected.

The contrast between this state of things and that which I have seen is most striking; and some of the principal points are these :—

(1) The changes that I have described, with the single exception of the first of those referred to under (*d*), have never been seen in Euglenæ when first brought from their original habitats. They have been met with only when the Euglenæ have been kept for variable periods in one or other unnatural set of conditions.

(2) Among the thousands of cases in which these various changes have been seen developing in Euglenæ, in no single instance has Cienkowski's initial stage of infection been met with—that is, in no instance have swarm-spores been seen adhering to the surface of the Euglenæ, as we should expect to have seen over and over again if their morbid states had been due to infection, and brought about in the manner that he describes, and which I have myself seen in certain Algæ.

(3) There is the further difficulty that almost invariably, in the cases which I have been recording, there has been a complete absence of any evidence of multiple infection, such as Cienkowski's observations would lead us to expect, if the sporangia found within the Euglenæ had been due to infection.

(4) Finally the sporangia found in these Euglenæ are very similar to those found within the thick and tough-walled resting-eggs of some Rotifers (such as I have represented in Pl. iv., figs. 36, 39); and also to the bodies into which the substance of the resting-spores of *Vaucheria terrestris* were resolved (as shown in Pl. ii., figs. 17, 18), every stage in the formation of which was observed—and with which, most certainly, infection had nothing to do.

(*g*) The Origin of Chlamydomonads from Euglenæ.

The transformation now to be described was observed in a portion of a Euglena pellicle which had been sent to me from the country, so that I am unable to speak as to the conditions to which the organisms had been previously exposed, though on

another occasion I saw a similar change occurring in Euglenæ which had been exposed to strong sunlight for a time in a shallow dish. In a day or two after the portions of pellicle above referred to had been received I saw the changes in question occurring, and other fragments of the pellicle were subsequently examined from time to time for about a week with similar results.

The Euglenæ were all encysted, and contained corpuscles of a dark green colour, though with an admixture of others that were colourless.

On examination the kind of appearance shown in Pl. xv., fig. 158, A (× 250) was seen, in which the sites here and there formerly occupied by Euglenæ now showed groups of eight to ten or more motionless Chlamydomonads contained within a softened and expanded cyst wall. A portion of this pellicle, more enlarged, is shown in B (× 375). The Euglena seems to divide into several segments while its cyst-wall softens. The resulting Chlamydomonads continue to divide in a pellucid jelly-like fluid so as to form much larger aggregates of small green nucleated cells such as are shown in C (× 375). After a time each of these units develops a red eye-speck, with a pair of flagella, and begins its active life.

There was no room for doubt that in the pellicle which I examined these bodies were formed from the Euglenæ, the early stages of the change being seen here and there replacing Euglenæ within their cysts in the otherwise even and uniform layer formed by these organisms.

(*h*) The Transformation of the substance of Euglenæ into one or more Amœbæ.

I have already described some modes of origin of Amœbæ from Euglenæ, but I have since seen them produced in two other ways.

In Pl. xv., fig. 159, A (× 375) some small encysted Euglenæ having very large eye-specks were beginning to become decolorised. There was liberation of brown pigment granules, and what was left of the green matter had become homogeneous. In the lower of the two specimens on the right the eye-speck had disappeared. In B (× 375) some of the Euglenæ are seen in different stages of decolourisation, while two of them had become completely converted into colourless protoplasm, and each had a large stationary vacuole near the centre. In this stage they were unquestionably Amœboid bodies, but it is by no means certain that they would have remained as such had they been left undisturbed. Bodies just like this, and produced by the transformation of a Euglena, sometimes develop into one or other form of Ciliated Infusoria as I have already shown (p. 110).

The other mode of transformation is to be seen in C (× 375), which is the representation of a very large Euglena that had been shut up in a small dark pot with very little air for three weeks. Its substance had broken down and become converted into five unequal Amœboid bodies, each of which contained a variable amount of a mixture of red, brown, and green matter in its interior. The envelope of this Euglena was quite thick though the organism had not become actually encysted. The Amœbæ seemed to have been formed in the same sort of way in which we have seen them produced within the closed cells of Spirogyra (Fig. 148).

In the last section I showed that other observers such as Nägeli, A. Braun, Pringsheim and others, had seen and described the production of lower organisms directly from the substance of Spirogyra resting-spores and filament-cells. They were so satisfied that they were not organisms which had obtained an entry from without, and that they were produced from the actual endochrome of the plant, that they regarded them as so many different kinds of spores or propagative cells destined to reproduce the plant. These investigators were able to produce no evidence in favour of this interpretation, however, and now after a lapse of fifty years their view is entirely discredited, and it is generally known that in the family to which this plant belongs "multiplication by zoospores does not take place." The facts observed by these distinguished investigators, however, remain, and they can only be regarded as so many instances of Heterogenesis.

Now, again, I may call attention to another and well-admitted case of the actual transformation of the contents of vegetable cells into Amœbæ—such as I have just described as occurring in Euglenæ, and have previously described as occurring in large Confervoid cells, and in the resting-spores of a small Spirogyra (pp. 3 and 10).

In the last edition of Carpenter on "The Microscope," edited by Dr. Dallinger (p. 556), there may be found the following important passage:—"Another phenomenon of a very remarkable nature, namely, the conversion of the contents of an ordinary vegetable cell into a free moving mass of protoplasm that bears a strong resemblance to the animal *Amœba,* has been affirmed by Dr. Hicks[1] to take place in *Volvox,* under circumstances that leave no reasonable ground for that doubt of its reality which has been raised in regard to the accounts of similar phenomena occurring elsewhere. The endochrome mass of one of the ordinary cells increases to nearly double its usual size; but instead of undergoing binary subdivision so as to pro-

[1] *Quar. Jour. of Micros. Science,* 1862, p. 96.

duce a zoösporange, it loses its colour and its regularity of form, and becomes an irregular mass of colourless protoplasm, containing a number of brown or reddish-brown granules, and capable of altering its form by protruding or retracting any portion of its membranous wall, exactly like a true Amœba. By this self-moving power, each of these bodies (of which twenty may sometimes be counted within a single *Volvox*) glides independently over the inner surface of the sphere among its unchanged green cells, bending itself around any one of these with which it may come into contact, precisely after the manner of an *Amœba*."[1]

In the same communication in which the above facts were recorded Braxton Hicks cited other observations showing a similar transformation of the cell contents of certain moss-radicles into Amœbæ. Speaking of these moss radicles he says,[2] "the endoplast of many of the elongated cells of which they are formed, not unfrequently detached itself from the contact of the cell wall, and collected into one or more ovoid masses of different sizes. These possessed all the optical properties of living, healthy vegetable protoplasm, which was made still more probable by the power it possessed in segmenting as gonidia do. By this process the whole of the cell of the radicle was empty, with, of course, the exception of these ovoid bodies. . . . Some of these masses changed their colour to red or reddish-brown; and gradually lost their colour till no trace of red or green remained, excepting reddish granules as in the case of the Volvox. These changes are shown in Figs. 6 and 7*a*. . . . These changes having proceeded thus far, they [the masses] gradually began to alter their form, and to protrude and retract processes exactly as Amœbæ, and as was noticed in the Volvox. They travelled up and down the interior of the cells, occasionally elongating themselves into almost a linear form. (See Figs. 7 and 9.) The movement of their contents presented the same phenomena as those of true Amœbæ. Although generally all the masses of green endoplast simultaneously underwent these changes, yet

[1] A production of Amœboid bodies has also been observed by Archer in *Stephanosphera pluvialis* (*Quar. Jour. of Micros. Science*, 1865, p. 117) though there was no decolorisation of the chlorophyll in them. This and other peculiarities seem to separate these bodies from the veritable Amœbæ seen by Braxton Hicks, and to make it possible that they were only unusual states of the cells of this Alga, and not heterogenetic products. If these cells reverted to one of the states of Stephanosphera, they would, of course, only be regarded as transitory phases in the life-history of the Alga.

[2] *Loc. cit.*, p. 97. Further on (p. 103) he says: "The circumstances under which the moss roots should be placed to show these phenomena is to float any common moss on a glass of water in the shade; and when the radicles they push out are of considerable length, they may be removed to the slide and examined. Most specimens, where not too much exposed to light and heat, will afford many instances of the above."

exceptions might now and then be found, either in the same cell, or in adjoining, where the changed and unchanged masses co-existed. The number of Amœboid bodies in each radicular cell would therefore seem to depend either upon the number of masses into which the endoplast is primarily divided, or upon the number of segmentations into which it again resolved itself. I have seen as many as seven in one cell moving freely about."

These are just such changes as I have often seen in cells of Spirogyra and other Algæ. But in this instance Braxton Hicks was able to carry his observations still further, and actually watch the development of the Amœbæ into embryo Ciliates. He says: "Anxious to learn what became of these bodies, I carefully watched one for some hours, and observed the following:—First, the movement by protrusion became gradually restricted till it was extinguished, the mass returning to the ovoid form it possessed originally. The exterior also seemed to become more rigid, although I do not think there was any distinct cell-wall. Secondly, the whole exterior became covered with very minute cilia, in constant vibration, by which the mass was kept in a state of agitation within the containing cell; the total motion was curtailed of course, but in bodies which I noticed moving in the water undistinguishable from them, the motion was rapid and rolling. Beyond this point I was unable to extend my observations on their life-history. These succeeding conditions are shown in Fig. 10; *c* represents the ciliated condition."

These observations of Braxton Hicks thoroughly accord with many that I have myself made, not only from the fact of the relationship that they reveal as existing between Amœbæ and embryo Ciliates, but also because they show what I have so often insisted upon, which is that Ciliated Infusoria do not, as a rule, grow up as such from small germs, but come into being almost of full size—though the size met with varies widely in accordance with the varying bulk of the matrices from which they take their origin.

This cumulation of more or less similar results arrived at independently by various workers, the importance of which has so often been inadequately realised, shows how full of wisdom was the prophecy made by Alexander Braun more than fifty years ago when he said, "The future will certainly unfold many interesting phenomena in this hitherto little-worked field."

LIST OF ILLUSTRATIONS.

THIRD PART.

A mere list of the Illustrations is here given, together with the Enlargements in Diameters of the several objects represented.

For the Description of the Figures the reader is referred to the text itself, at the pages indicated here, and on the plates after the numbers of the respective figures.[1]

PLATE XII.

[1] The essential parts of the different Photographs have been cut out and placed side by side in groups. The Plates in this Third Part are, therefore, really made up of 208 figures taken from separate Photomicrographs.

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BY

H. CHARLTON BASTIAN, M.A., M.D., F.R.S.

FOURTH PART.

XIX. ON VARIOUS HETEROGENETIC CHANGES OCCURRING WITHIN THE CELLS OF DIFFERENT SPECIES OF NITELLA.

THE following observations have been made upon *Nitella flexilis*, *N. opaca* and *N. translucens*. The former plant I have worked with principally, but during the last three years I have been unable to obtain specimens, as this species is very inconstant in its habitats. Where it occurred abundantly four and five years ago, I have more recently been unable to find a vestige of it. *N. opaca* and *N. translucens* on the other hand have been found year after year in the same sites. The former is considered by some to be merely a fertile form of *N. flexilis*; as I have seen it, however, it has always been a notably smaller plant, while the chlorophyll corpuscles are distinctly larger than those of *N. flexilis*. Its cells, in addition to being rather more opaque, are apt also to be more abundantly covered with Diatoms and other Algal epiphytes. *N. translucens* is also less satisfactory for examination and for experimental purposes by reason of the large size of its cells, so that use can only be made of the comparatively young shoots.

The Contents of Nitella Cells.

There are three respects in which the cells of *N. translucens* differ from those of the other two forms. In the first place its chlorophyll corpuscles are distinctly smaller even than those of *N. flexilis*; and in the second place floating on the surface of its circulating slime, and moving therewith, are multitudes of amorphous bodies of varying size and shape, like stones that may be picked up on a macadamized road. These are shown in

Pl. xvi., figs. 160, A (× 125), and 168b, F (× 250). In *N. flexilis* and *N. opaca* the representatives of these latter bodies are spheres of different sizes having an appearance very like the flower heads of the common Bur-dock, such as are shown in Fig. 160, B (× 375), and 168b, D (× 375). By the use of a dilute solution of iodine I first ascertained that each of these stone-like particles and bur-like bodies is contained within a much larger hyaline envelope whose walls are so delicate as only to be made out with difficulty. One of these hyaline envelopes with its contained concretion which had been expressed from a small cell of *N. translucens* is shown in Fig. 160, C (× 375). The larger bur-like bodies can also occasionally be seen, within cells which are more than usually transparent, to be contained within similar hyaline envelopes, though I have never seen the envelopes around these bodies preserved, amid the expressed contents of cells of *N. opaca* or *N. flexilis*.[1] These envelopes as seen through the cell walls are shown in Fig. 168b, A, B (× 250).

Taking part in the cyclosis within the cell, and intermixed with the concretions above referred to, there are a multitude of other more obvious hyaline spheres, such as are shown in a cell of *N. flexilis* in Fig. 160, D (× 250).[2] These spheres are most abundant in *N. opaca*. They are almost as numerous in *N. flexilis;* but are generally not discoverable in *N. translucens.* They seem always to be most plentiful in young, actively growing cells. They are well seen when the contents of cut cells are pressed out by the mere weight of a cover glass in a drop of a solution of eosine (Fig. 168a, A, × 250). They appear to have much the same constitution as soap-bubbles, and to be devoid of contents, though, as I shall subsequently show, this is not the case. They vary much in size in every cell.

A quantity of finely granular protoplasmic slime is to be seen circulating in the cells of each species on the inner surface of the chlorophyll layer, between it and the other contents above referred to. In the midst of this slime there are a number of small protoplasts of a spherical or ovoidal shape. They are unrecognizable in the circulating slime, but are always to be found when, by exerting much pressure, a cell has been burst

[1] Since writing this I have many times seen these bur-like bodies within the hyaline envelopes, when the contents of small cut cells have been pressed out beneath a cover glass in a drop of a 2 per cent. solution of eosine (Fig. 168b, E), and have found that they can be made easily visible, within the smallest cells, by running a drop of a $\frac{1}{12}$ per cent. solution of osmic acid beneath the cover glass.

[2] This photograph was taken after the cyclosis had been stopped by running under the cover glass a drop or two of a weak solution of formalin ($\frac{1}{2}$ per cent.). In about five minutes cyclosis was arrested. After a time it recommences, if the cell is placed in fresh water and has not been too long exposed to the formalin.

beneath a cover glass either in a drop of distilled water, or of a very weak solution of gentian-violet or other dye.[1] These plastides are shown in Pl. xvi., figs. 167, 168, and more details will be given concerning them farther on. They are the only formed elements to be found in the slime.

It occasionally happens, however, that portions of the slime become separated from the general mass and aggregated into spheres of different sizes such as are shown in Figs. 160, E, and in 168b, C (× 80). These spheres are a little more densely granular than the general substance of the slime, though they move with it around the cell—often rotating on their axes at the same time.

The various contents of the Nitella cell that I have been enumerating circulate, as I have said, within the chlorophyll layer, which lies on the surface of the primordial utricle lining the thick outer wall of the cell. The primordial utricle would seem to be represented by semi-fluid protoplasm : there is certainly no separable membrane. The chlorophyll corpuscles are for the most part regularly disposed in spiral lines, as may be seen in a portion of one of the cells of *N. translucens* in Pl. xvi., figs. 161, A (× 100), and they are more or less ovoid in shape. In rather unhealthy cells, however, various alterations may be met with. Thus sometimes they may be very irregularly disposed, circular and more sparsely distributed, as shown in B (× 250) from a cell of *N. opaca*. At other times, as in another old, pale cell of the same form, the corpuscles may be very irregular in size and shape, and some of them may be seen undergoing processes of fission (C, × 250). In many cells again the chlorophyll corpuscles may be more or less filled with starch, beginning in the form of minute granules (as in D, × 500), which ultimately increase greatly in size and number. Larger grains which have been stained with iodine are shown in some corpuscles expressed from a cell of *N. flexilis* (E, × 375) ; and still larger scale-like ovoidal grains distending other corpuscles are shown in F (× 375) from an older cell of *N. opaca*.

(*a*) On the most Common of the Heterogenetic Changes occurring in Nitella Cells: the Origin of a Pseudospora.

When specimens of either of these species of Nitella are brought home from the ponds, placed in fresh tap water and kept indoors, after a week or less some of the cells will be found to have become more or less discoloured—that is, they will have lost their bright uniformly green colour, and may be seen by the

[1] So much pressure is required to burst one of these cells, that a No. 2 cover glass should be used, and the pressure made over a large surface by means of a small cork.

aid of a lens to have assumed an irregularly spotted appearance, or to have become of a more or less uniform drab colour.

When these altered cells are examined under low powers of the microscope they present such appearances as are shown in Fig. 162. In A (× 25) we have one of the very common appearances presented by cells of *N. flexilis* in which they are crowded with what we shall find to be Amœboid organisms in different stages of development. In B (× 12) we have a terminal bunch of young cells all showing similar changes. At times, however, we may see a group of such young terminal cells perfectly healthy, though the cell with which they are connected may be in the final stages of change. In C (× 75) a cell and two portions of *N. flexilis* are shown under a rather higher power; the entire cell was fairly healthy, and lined with green chlorophyll corpuscles; the cell below contained large Amœbæ gorged with chlorophyll corpuscles, while the light cell above was densely packed with small specimens of Actinophrys.

Two other less common kinds of change are shown in Pl. xvi., fig. 163, which are not unfrequently to be found in association with the preceding—that is, in contiguous cells. In A (× 25) the Amœbæ are of the kind previously met with, only in this cell they had nearly all passed into a peculiar kind of resting stage. In B (× 25) two of the cells (a large and a small one) contained the common large Amœboid organisms, while the cell below and that by their side had undergone a very different kind of change. These two cells, instead of being lined with a continuous layer of minute chlorophyll corpuscles, were lined with a continuous layer of minute Amœbæ, such as are shown under a high power in Pl. xvii., fig. 172, B (× 375). These latter changes will be considered later, after I have first dealt as fully as possible with the more common kind.

When the common change is in an early stage of progress the Nitella cell presents, on microscopical examination, the appearances shown in Pl. xvi., fig. 164, A (× 375) more or less throughout its whole length. This was a healthy cell of *N. flexilis* two days previously; but now, as shown, the chlorophyll corpuscles are disarranged; there are irregular gaps between them, and intermixed are what appear to be mere motionless aggregates of chlorophyll corpuscles. At a later period, as in B (× 200), which represents a portion of a cell from a fresh stock of *N. opaca* that had been in distilled water for two days, no free chlorophyll corpuscles were to be seen; all had disappeared, and in their place there were a number of green spherical masses in many of which the individual chlorophyll corpuscles were quite distinguishable, though in others they were not. These spherical bodies were not quite motionless, but their movements were excessively slow and difficult to distinguish, as may be gathered from the

fact that the photograph was taken with an exposure of one and a half minutes, and without the previous use of any reagent to check or arrest their movements. The next change is shown in C (× 200) which happens to be a portion of a cell of *N. translucens*. The masses are now completely motionless, spherical, and with fused contents, varying in colour from dark green to olive-brown, while a narrow rim of slightly brownish protoplasm is to be seen at the periphery. Later, as in D (× 375), this peripheral rim of protoplasm becomes wider and more completely colourless, while indications of its commencing segmentation are to be seen in some of the spheres.

The later stages in the life of these spheres is represented in Fig. 165, in which A (× 400) shows the process of segmentation of a large mass of colourless protoplasm into nucleated Monads, while a large spherical mass of pigmentary residue is left; B (× 375) shows segmentation of another sphere into small Amœbæ several of which, larger and set free from an adjacent sphere, may also be seen; C (× 200) shows a sphere which has undergone segmentation almost throughout into a multitude of Amœboid corpuscles, leaving only residual matter scattered between them; while in D (× 400) a sphere of the same kind is shown partly empty, but containing a large olive-brown residual mass together with a number of Amœboid corpuscles.[1] All these specimens were from cells of *N. flexilis*.

The size of the Amœbæ produced from segmentation of the spheres varies a good deal. Those seen in Fig. 165, B (× 375) are of about the usual size, but those shown under a lower magnification in Fig. 166, A (× 225) were unusually large. Some of them were exhibiting sluggish movements. The spheres in which they had been produced had extremely thin bounding membranes—so thin that they soon completely disappeared. Much variation exists in this respect as several of the figures will show. Amœbæ are generally rather more common than Monads as products of segmentation; but at times the latter may be seen to predominate. Some of these Monads are shown in Fig. 166, B (× 450) which, before the use of a weak osmic acid solution, exhibited slow movements by means of a rather long single flagellum.

Thicker envelopes of the spheres may at times be seen, with all the products of segmentation gone, and containing only a sharply defined residual mass of undigested pigmentary matter, as in C (× 375). And in cells where the chlorophyll corpuscles,

[1] These are such bodies as have been seen many times before in other transformations. Under suitable conditions they become fluent active Amœbæ. It is a primary "resting stage" assumed at birth, or after some growth as a stationary corpuscle, but always anterior to an active stage.

on which the Amœbæ have fed, have been filled with starch grains, such empty cysts may be found as are shown in D (× 375) —that is, with starch grains adhering to their outer surface.

(*b*) Some Data as to the Times occupied by the foregoing Changes and on Modes of inducing them at Will.

A few extracts from my notebooks will suffice to throw light upon this subject.

(1) A small healthy verticel of *N. flexilis* was placed in a small corked tube with tap water, which was then put into a dark cupboard at a temperature of 60° F. On examination after two days, cyclosis was found to be still continuing in five of the rays, but in the sixth, composed of two cells, it had stopped. In the proximal cell, which was the least altered in appearance, the chlorophyll corpuscles had lost their regular arrangement, but only slightly. Many of the corpuscles were turned edgeways instead of lying flat. In the more altered and shorter terminal cell of the ray the disposition of the chlorophyll corpuscles was completely changed. They now formed a very irregular network over the whole surface of the cell.

After a brief examination the verticel was replaced in the corked tube, and again put into the cupboard the temperature of which was then 68° F. Ten hours later there was still no appearance of spheres within the proximal cell, but in the terminal cell all the chlorophyll corpuscles had become most irregularly disposed.

Exactly twenty-four hours after the last examination, the whole of the contents of both cells were found to be contained within spheres of different sizes. Those in the terminal cell were rather more advanced in development than those in the other—many of them having rims of colourless protoplasm, while the central parts were yellowish rather than of an olive green colour as in the proximal cell.

The complete apparent transformation of the whole of the contents of the cells into Amœboid spheres, whose active stage was completed, and in which digestion of the food with which they were gorged was well advanced, was therefore brought about in the space of twenty-four hours.[1]

(2) Some observations on another verticel of the same stock, made at the same time, and which had been treated in a similar manner, will give the time occupied by the later stages.

At 11 a.m. one morning several cells of this verticel were seen in which complete conversion of the contents into spheres

[1] Many of the bur-like bodies are swallowed, and so, in *N. translucens*, are the great majority of the analogous amorphous concretions.

had taken place, and these were showing the first stage of discolouration—many being of a dark olive-green colour, but with the outlines of the contained chlorophyll corpuscles still distinct.

At 3 p.m. Discolouration had progressed rapidly, as by this time most of the spheres were of a yellow olive colour, and some were showing a narrow colourless border.

At 9 p.m. Great numbers of the spheres had undergone almost complete segmentation into Monads, others had partially segmented, and others again had not actually commenced to segment.

Thus from the first stage of discolouration of the spheres to their actual segmentation into Monads only about ten hours is needed.

(3) Two very small verticels of *N. flexilis* containing twelve healthy cells, in each of which there was an active cyclosis, were exposed late one night for the space of an hour to strong light concentrated from a microscope lamp.

The next morning (September 15) at 9.30 a.m. I found that in all but two of these cells the cyclosis had stopped, and the chlorophyll corpuscles were more or less disarranged.

As I wished to ascertain whether, when previously healthy cells were killed in this way, they would undergo the usual changes, they were placed in a covered watch glass on the mantel-piece in some fresh tap water.

By 5.30 p.m. on the same day cyclosis was still continuing in the two cells. Several of the other cells showed the first stages in the formation of the spheres. By 11 p.m. the spheres had grown much in size and in number.

September 16, 9.30 a.m. The formation of spheres was more or less advanced in all the ten cells. Some of them were about to segment into Monads; and nearly all of them were more or less discoloured.

September 17, 9.30 a.m. In all the ten cells the spheres were by this time going through the usual segmentation into Monads or Amœbæ. Active cyclosis was still continuing in the other two cells.

Sometimes placing healthy cells of a fresh stock in distilled water will cause their speedy death and the formation of spheres throughout their whole length. The specimen shown in Pl. xvi., fig. 164, B is a portion of a cell of *N. opaca* which had been in distilled water only two days. It had probably not been killed till some time during the second day. At other times death of the cell takes place more rapidly. Thus a cell of *N. flexilis* was examined and found to be healthy with active cyclosis going on. It was then transferred from New River water to distilled water, and fifty-four hours later the whole cell was full of developed spheres showing early stages of segmentation.

I have also tried to determine these changes at will in one or other of the *Nitellæ* by wounding the primordial utricle and chlorophyll layer without breaking the wall of the cell. This is a matter of some difficulty; the elastic tension of these cells is so great that very strong pressure may be made upon them before the cell is ruptured, and yet strong pressure is required in order to damage the contents of the cell. If a needle be laid across the middle of a cell, for instance, and pressed firmly the free half of the cell will often come up almost to a right angle with the other half before rupture occurs. When the pressure has been short of producing rupture, but the chlorophyll layer has been broken, shock causes the cyclosis to stop for a time. In a cell of *N. translucens* in which I had made two ruptures of the chlorophyll layer close to one another, one larger than the other, I found on the following day cyclosis restored. The smaller damage did not obstruct the current, but the larger caused the cyclosis to be in part divided at the point of injury—as if there were two distinct cells—though in part the current passed it. On the following day, however, this cell was found to be dead, and in the early stage of sphere-formation throughout.

Cutting through a cell in tap water leads to uncertain results. Sometimes I have found when a verticel has been cut off, leaving a long portion of the next cell in connection therewith, that after twenty-four hours all the chlorophyll corpuscles in this cut cell have been aggregated into spheres of different sizes. On other occasions, though the cut cell does not show changes of this kind, previously healthy cells growing from its extremity may be found in two or three days to be full of spheres in different stages of development. I may cite as an example from my notebook the following case: "A healthy-looking terminal portion of *N. opaca* was cut off and placed in a small covered pot containing equal parts of tap and distilled water. The pot was then placed on an incubator at 72° F. After forty-eight hours the cell which had been cut through was still green though the chlorophyll layer had in part shrunk away from the cell wall and multitudes of bacteria were seen in this situation. There were, however, none of the ordinary spheres, and no other organisms of any kind. On the other hand, every one of the ten or more closed and previously healthy cells was by this time filled with spheres whose contents were in the olive brown stage of discolouration, some of them showing colourless protoplasm at the periphery either unsegmented or in process of segmentation—though none of the Monads or Amœbæ were as yet free. Not a single free chlorophyll corpuscle was to be seen, all had been devoured.

Thus in many cases by one or other of these methods one is able to determine at will in previously healthy cells, their death and the speedy supervention of the remarkable changes which I have described above.

(*c*) On the Mode of Initiation of the Foregoing Changes. Are they due to Infection or to Heterogenesis?

In regard to the precise mode of initiation of these changes much obscurity at first exists. An observer may study them for a long time and fail to make out what are the earliest stages of the change. All that is obvious I have already referred to. We seem to have first an arrest of cyclosis, then more or less disarrangement of the corpuscles in the chlorophyll layer, followed in the course of a few hours by the appearance throughout the cells of more or less spherical aggregates, varying in size, of what often appear to be unaltered chlorophyll corpuscles.

But a more thorough search through cells in one of the earliest stages of change to which I have referred will reveal a number of delicate, almost motionless Amœbæ, having about two or three times the diameter of the chlorophyll corpuscles, either empty or containing one, two, or three of these corpuscles in their interior, as in fig. 167, A (× 375). This figure shows some of the expressed contents of a cell of *N. flexilis* in which cyclosis was active forty-eight hours previously. The chlorophyll corpuscles become digested, the Amœba rapidly grows, and soon becomes completely enveloped by other corpuscles, which seem to be attracted, or to adhere to its external surface. Often, however, it happens that the young Amœbæ do not become thus completely enveloped and obscured by adherent chlorophyll corpuscles from the first, as is shown in Fig. 167, B (× 200).

It may be taken, therefore, as certain that these common changes within Nitella cells begin by the simultaneous appearance throughout their whole length of multitudes of minute Amœboid organisms. The question therefore naturally arises, Whence do they come? Are they produced by heterogenesis from some of the normal constituents of the cells? Or are they parasites which have obtained an entry from without? We are again brought face to face with this oft-recurring problem.

What is to be said for or against the hypothesis of Infection?—None of these Amœbæ are to be seen smaller than $\frac{1}{4000}$ or $\frac{1}{5000}$ of an inch in diameter. Their movements are so sluggish as to be scarcely appreciable even under high powers of the microscope. They do not even show changes in shape; and they are never seen to undergo a process of fission.

None of such Amœbæ are to be discovered in the tap or distilled water outside, and none are to be seen adhering to or attempting to penetrate the thick walls of the Nitella cells. Yet within thirty-six hours or less from the death of the cell—that is from the arrest of cyclosis—the cell may be found to swarm throughout more or less of its length with these minute Amœbæ,

which have already begun to devour and surround themselves with chlorophyll corpuscles. If the Amœbæ had come from without, as they cannot be seen to multiply by fission, a whole army of them should be distinguishable on the walls of each cell, and attempting to struggle through its substance. But absolutely nothing of this kind is to be seen. The Amœbæ and the Monads that have been shown to be produced by the segmentation of the spheres which so rapidly grow and develop within the Nitella cells are comparatively large organisms. So that if they attempted and were able to penetrate the walls of the Nitella in numbers they would, of course, be always easily recognisable. But the slightest indication of such a process, or even of such bodies in numbers outside the cells, may, as I have said, be looked for in vain.

Nor are there any recorded cases in which multitudes of minute Amœbæ have ever been recognized simultaneously making their way into vegetable cells. The case of Vampyrella is wholly different; in which a single large active organism penetrates the much thinner wall of a Spirogyra cell by a projection of its body substance—after contact for a time, and a probable partial solution of the cell wall by some excreted cytase. But to suppose that multitudes of small almost motionless Amœbæ such as may be seen to give rise to the large rapidly developing spheres, could penetrate the comparatively thick, and previously unaltered wall of a Nitella cell, almost passes the bounds of credibility—while to suppose that they could do it in numbers, within the space of thirty-six hours or less, and yet that not a trace of any such process is to be seen, is too absurd a notion to be seriously entertained.

It might, perhaps, be thought by some that a few of these Amœbæ only may have entered after the death of the cell and then rapidly multiplied therein. This, of course, does not get rid of the difficulty of the perforation of the thick wall of the Nitella cell by such organisms, and it is confronted by another difficulty, to wit, that Amœbæ never multiply rapidly by fission.

Nor, with a view to get over the difficulty that neither Monads nor Amœbæ are to be seen in numbers penetrating the Nitella cells, either during life or after their death, can it be supposed that they penetrate as very minute scarcely visible organisms. The difficulty of penetration of the thick and unaltered wall of the Nitella cell would remain as before; and, moreover, such a supposition is negatived by the fact that the products of the segmentation of the spheres are Amœbæ or Monads of comparatively large sizes, such as are shown in Figs. 165 and 166.

Everything, therefore, is opposed to the notion that the changes I have been describing within Nitella cells are due to infection, and the invasion of parasites from without similar to

those that are so plentifully produced within the cells by the segmentation of Amœboid spheres.

What is to be said in favour of the Heterogenetic origin of the Amœbæ?—All the facts above mentioned that tell so strongly against the hypothesis of Infection must be considered to favour the only other possible mode of accounting for the presence of the multitudes of minute Amœbæ within the recently dead Nitella cells — namely, the view that they are heterogenetic products.

In further support of this view I am able to record other confirmatory facts.

I have ascertained that a dilute solution of aniline blue (three minims to two drachms of distilled water) is so little harmful to minute Amœbæ, Ciliates and Rotifers that they will live in it for some days; but if cells of Nitella are immersed in it for a short time they die after some hours, and then never undergo the usual changes.

In illustration I will make the following quotations from my note-book, made on October 26, 1898:—"Nine days ago another healthy terminal tuft of *N. flexilis* was allowed to remain in the aniline blue solution for one and a half hours. At the end of that time cyclosis was still continuing. The tuft was then transferred to fresh water in a covered watch glass, and the next day cyclosis was found to have stopped in all the cells. Since, although the chlorophyll layers have broken away from the walls of the cells, in not one of them is there any evidence of the formation of spheres or other of the usual changes." On the other hand, six days previously I had made the following entry as to the influence of some of the same aniline blue solution on animal organisms:—"When filaments of Vaucheria have been mounted in it [beneath a large cover glass] Rotifers, Ciliates, Amœbæ, Monads and Bacilli seem to be unaffected, although portions of the contents of the Vaucheria become deeply stained. When such specimens are surrounded by vaseline some of the other organisms live for several days, and the Bacilli for a week or more."

It would seem, therefore, that dilute solutions of this stain, which is comparatively harmless to minute Amœbæ and other animal organisms, exercises a distinctly noxious influence upon the vegetable protoplasm, so that when the cell dies, the swarms of minute Amœbæ are no longer produced, and all the ordinary changes are absent. Some slight alteration in the vegetable protoplasm produced by the stain checks what appears to be a customary heterogenetic process therein, although the agent that produces the change is comparatively harmless to minute Amœbæ and other animal organisms.

But, it may now fairly be asked, can you come any closer to some actual proof of the truth of your view that the minute Amœbæ found in such numbers within recently dead cells of Nitella are in reality heterogenetic products? To which I can reply in the affirmative, since I can show that there exist normally within the healthy cells of Nitella two kinds of elements which may be capable of giving origin to the Amœbæ. There are (*a*) multitudes of tissue elements, mere plastides, numbers of which are of just the same size as the Amœbæ that begin to devour the chlorophyll corpuscles; and there are (*b*) what I have hitherto spoken of as 'hyaline spheres,' which exist in large numbers, and some of which can be shown to undergo most significant and remarkable changes. The nature of each of these elements and the changes undergone by them require to be carefully considered.

(*a*) The plastides to be found in the Nitella protoplasm I have already referred to (p. 246), but details were purposely reserved for the present occasion.

For a long time I knew nothing about these plastides, as they cannot be recognized within the living cells; and they are, moreover, never to be seen among the contents of the cells undergoing the changes now under consideration. My first acquaintance with them was made by bursting small Nitella cells, free from saline depositions, in a drop of a weak solution of gentian-violet; by means of firm pressure over a thick cover glass. Among the expressed contents of the cells these plastides speedily became obvious, and distinguishable from the scattered chlorophyll corpuscles and starch grains by the delicate rose tint that they assumed. After working for a time in this way I found a far better method for studying these and other contents of Nitella cells. This consisted in cutting with small scissors the delicate and minute terminal Nitella cells (which are always the most prone to undergo the changes under consideration), dropping the cut portions into a small drop of a two per cent. solution of eosine, and gently applying a cover glass. The mere weight of the cover glass causes some of the contents of the cells to be extruded, with an immediate red staining of the bur-like or stone-like concretions and also of the plastides; while crowds of the delicate hyaline spheres become visible as colourless or faintly blue spaces in the midst of the pale red ground afforded by the eosine solution.

The plastides are spherical or ovoidal in shape, as may be seen in Fig. 168 A (× 375), but they vary very much in size in the same cell, and often still more in different cells. My observations concerning them have been made upon *N. flexilis* and *N. translucens;* principally the former, as the plastides in this

species are notably larger than they are in *N. translucens*, one of which, undergoing fission, is shown in E (× 375).[1]

My impression is that these plastides tend to be rather larger and more numerous in young than in old cells. In their ordinary condition they contain nothing but the finest granules; no trace of a nucleus is usually to be detected, though they may, not infrequently, be found elongated and in different stages of fission, as may be seen in C (× 375) and in D (× 250). In C there were appearances somewhat suggestive of a nucleus in each half, though none is to be seen in B (× 375). The way in which these plastides originate is obscure. It would seem probable that they are born in the protoplasmic slime in which they are found, in much the same way that the chlorophyll corpuscles are born in the outer stationary layer of protoplasm.[2] I have not been able to identify any of them smaller than about $\frac{1}{5000}$ of an inch in diameter. In sizes beneath this they seem indistinguishable from other particulate contents of the cell; and the same may be said concerning the beginnings of the bur-like bodies, which are almost equally numerous.

In some cells, most of the plastides are found to be enclosed within a delicate envelope, as in J (× 375); while in other cells some of the plastides contain a varying number of small irregularly shaped bodies, of uncertain nature, in the midst of their usual merely finely granular protoplasm. Two plastides in which such bodies were larger than what I have hitherto seen are shown in H (× 375). More commonly several minute circular specks or rings only can be made out in their interior, while in E bodies of an intermediate kind are shown.

Among the expressed contents of Nitella cells one may often find one or more chlorophyll corpuscles adhering to the surface of the plastides, as may be seen in Pl. xvi., fig. 167, C, D, E (× 375). These specimens were expressed from a cell of *N. flexilis*, and stained with gentian-violet. Their similarity in size to that of the Amœbæ shown in Fig. 167, A, is notable. It is also noteworthy that no nuclei are commonly recognizable in these initial Amœbæ, though a reference to Fig. 165 will show that they are always most distinct in the Monads or Amœbæ, into which portions of the Amœboid spheres ultimately segment. They appear in their peripheral colourless protoplasm, anterior to the occurrence of segmentation (as in Fig. 164, D), in much the same way that nuclei appear in the sporangia of *Polyphagus Euglenæ* and in other cases.

[1] During the time that I have been working at this part of the subject I have been unable to obtain any *N. opaca;* the pond in which I have found it for four or five years being now overgrown with coarser weeds.

[2] It is interesting to note that chlorophyll corpuscles also occasionally undergo processes of fission (see Pl. xvi., fig. 161, c), though they, like the plastides, are also devoid of a nucleus.

The possibility that the initial Amœbæ might be derived by a process of transformation, or in some way take their origin from these normal constituents of the Nitella cells seemed for a time to be strengthened by work carried out with the aid of the eosine solution.

I thus ascertained, for instance, two important points:—(1) that as soon as the Amœbæ appear within the Nitella cells, or very soon after, the plastides are no longer to be found; and (2) that the minute Amœbæ may at times appear in the dying cell before its cyclosis becomes arrested.

(1) This disappearance of the plastides, as the Amœbæ appear upon the scene, together with the fact that the former are unrecognizable while still within the healthy living cell, accounts for the fact that for a long time I knew nothing about the existence of these plastides. As I have already stated, in the expressed contents, as well as in that remaining within cut healthy cells, the bur-like bodies and the plastides become at once recognizable by their red staining by eosine. But in cells that are undergoing the changes we have been considering, while eosine will reveal plenty of the bur-like bodies among the Amœbæ, none of the plastides are ever to be seen, except occasionally during the very early stages of the change.

(2) I recently took some cuttings from *N. flexilis*, which had been in New River water for a fortnight and presented a most healthy appearance, and placed them in a closed pot with distilled water. After forty-eight hours I took some of the cells which, on examination with a hand lens, had a perfectly healthy appearance; I cut them in drops of the eosine solution, gently expressed their contents, and subsequently examined them microscopically. In some of the cells, and among their expressed contents, the red plastides were seen in abundance, but none of the small Amœbæ could be detected. In other cells only a few of the plastides could be found, but numbers of the minute Amœbæ were seen, some of which were large enough and had actually begun to swallow the chlorophyll corpuscles.

I then first examined some cells in distilled water; and in two or three of those in which cyclosis was still slowly continuing, I found subsequently, on cutting such cells in eosine, that they contained numbers of the minute Amœbæ, but only a small number of plastides. This was quite a revelation to me, as I had always previously supposed that the cessation of cyclosis preceded the appearance of the initial Amœbæ; while, as a matter of fact, the change at times seems to commence when there is not only no appreciable alteration in the appearance of the cells under examination by a hand lens, but when, even under the microscope, nothing very unnatural may be detected.

The mode in which, if it occurs, the Amœbæ are produced

from the plastides I have been unable to follow. It cannot be that the plastides are in all cases bodily transformed into the Amœbæ. Some of the former are very large, as in B (× 375); and as a rule the average size of the young Amœbæ which so soon appear in large numbers within the dying cells is distinctly smaller than that of the plastides whose place they take. There are two possible modes in which the plastides might be transformed and give origin to the Amœbæ. Either fission might occur associated with molecular transformation, or the Amœbæ might, in some way, be produced in the interior of the plastides, and the changes to which I have previously referred, and which are represented in Fig. 168, E and H, may be the beginnings of such a process.

The first of these modes would seem to be the simpler process, but mere fission alone would not be adequate; it must be associated with some molecular transformation leading to increased vitality; and the change (if change it be) is shown by the fact that these minute initial Amœbæ, while living, differ from the plastides in remaining unstained by the eosine. It is perfectly certain that in the healthy Nitella cell these plastides contain only the finest granules, as in A and B; on the other hand they are occasionally met with in which the granules are altogether larger and more like those found within the young Amœbæ. Such specimens are shown in Fig. 168, F (× 375) and G (× 700). Each of these specimens came from older cells of *N. flexilis*, in which the chlorophyll corpuscles were loaded with large starch scales, two of which, stained purple with iodine, are to be seen in part in G. Then, again, the second supposition above made is based upon the fact that some unusual process is evidently occurring in the plastides to which reference is made. These changes have occasionally been very common in the plastides from some cells; while in those from ordinary healthy cells such appearances are never to be met with since the protoplasm of their delicate plastides contains only the finest granules.

Though I at first thought that the Amœbæ might take origin from these plastides in one or other of the ways indicated I have, after careful search, found nothing further in support of this view, and have gradually relinquished it as the evidence has become more clear that they are produced in a different manner, now to be described.

(*b*) What I have hitherto spoken of as hyaline spheres I find referred to in the following terms by Sachs.[1] After speaking of the disappearance of the nucleus of the cell and the formation of grains of chlorophyll, he says: "With the growth of the whole

[1] "Text-Book of Botany," Trans., 1875, p. 286.

cell these grains also grow, and multiply by repeated bipartition; they adhere to the inner side of the outermost thin stationary layer of protoplasm, and take no part in the rotation of the layers which lie further inwards. The rotating protoplasm becomes differentiated, as the cell grows, into portions, some very watery and others less watery and denser, the former appearing as hyaline cell-sap in which the latter float in the form of roundish larger or smaller lumps."

It is important to recognise that they are "lumps" of hyaline protoplasm bounded by a thin limiting membrane, and that they are not mere hollow spheres. They vary in size from minute globules $\frac{1}{10000}$ of an inch up to spheres as much as $\frac{1}{800}$ of an inch in diameter, though they do not attain so great a size as the spherical envelopes of the bur-like bodies shown in Fig. 168b.[1]

In healthy cells the protoplasm of the spheres seems quite structureless, showing no granules of any kind in their interior, though it is quite common for a few granules to be found adhering in one or more situations to their exterior (Fig. 168a, A, × 250). But as the vitality of the cells diminishes more and more of these spheres will be found to show a number of fine granules, or fine mixed with coarser granules, in their interior. The granules increase in number till at last the spheres become filled with them, as in the body shown within the cell in Fig. 168a, C (× 250). When a cell containing a number of these modified hyaline spheres is cut and allowed to fall into a drop of a 2 per cent. solution of eosine, we find that most of the spheres show a structureless appearance, and, owing to the existence of a limiting membrane, they may remain in the eosine solution even for three or four hours without becoming stained internally. In B (× 375) the large colourless sphere on the left contains structureless protoplasm, while those on the right show granules beginning to appear in their substance. In D and E (× 375) the granules, fine or coarse, are distinctly more numerous. These granules later on, as they become more abundant, often show slight movements like those in the substance of an Amœbæ. I have seen one of these colourless bodies burst in the eosine solution, with immediate staining of the granules issuing therefrom. When three or four large granules first appear, they are commonly motionless, owing perhaps to the protoplasm being then more viscid.

But in addition to the movements of the granules these altered spheres show slight changes in shape, and I have seen them undergo fission both within the cell and outside in the eosine solution—especially in the latter, which, as I have long known, at first stimulates the movements of Amœbæ, though after

[1] These bur-like bodies are referred to by Sachs as "bodies of globular shape covered with delicate spines, consisting also of protoplasm."

a comparatively short time it kills them and they then become stained of a red colour. One of these Amœboid bodies thus killed and stained is shown in B below the two large spheres on the left hand; while one of the products of fission still remaining unstained in the eosine is shown in F (× 375), and still smaller products killed and stained in G (× 375). These latter are the ultimate products of segmentation, which appear as young Amœbæ having no visible nucleus. They speedily begin to swallow the chlorophyll corpuscles, and thenceforth grow rapidly; the labours of digestion putting a stop for a time to further processes of fission. During these changes the unaltered hyaline spheres seem to become dissolved and speedily disappear.

After prolonged and careful study of these changes in Nitella cells I am, therefore, firmly persuaded that the multitudes of small, almost motionless, Amœbæ, which appear in such numbers in dying and recently dead cells, do not, for the reasons already stated (p. 253), come from without by any process of infection. The fact that the initial non-nucleated Amœbæ found in the Nitella cells are totally different from the nucleated Amœbæ into which the spheres segment (Pl. xvi., figs. 165, 166); and the fact that multitudes of such comparatively large bodies can never be seen adhering to and making their way through, the thick walls of the Nitella cells are two of the principal difficulties standing in the way of the infection hypothesis. But these difficulties receive a ready explanation on the supposition that the small initial Amœbæ, as they first appear, are derivatives from the modified hyaline spheres which begin to show themselves within the Nitella cells as their death approaches.

(*d*) Concerning a Modification of the Organism previously described; answering to what is commonly known as a Resting Stage.

Two cells of *N. flexilis* in which the spheres were almost all of the kind I am now about to describe, though mixed with them were some of the ordinary form, are shown under a low magnification in Pl. xvi., fig. 163, A. And in Fig. 169 A (× 75) a portion of another cell is shown under a higher magnification containing similar bodies, varying much in size; while in B (× 375) some of these bodies are to be seen much more highly magnified and still varying greatly in size. In C (× 375) a larger specimen still is to be seen in its fully developed condition, showing a homogeneous sphere of glistening protoplasm within a thick inner envelope. In one respect this specimen presents a very unusual character in the form of a crowd of very delicate filiform processes springing from the surface of the inner envelope. Such processes have occasionally been met with in other

organisms, but they have only been noticed in one batch of these resting forms.[1]

It will be seen that in these particular spheres instead of segmentation taking place at the periphery into Amœbæ or Monads, the enveloping membrane usually becomes notably thicker and there is formed within a second envelope, mostly ovoidal in shape (though appearing spherical in some aspects), and within this again a spherical mass of glistening or refractive protoplasm, which is itself bounded by a distinct membrane.

Abortive or irregular forms of this resting phase are not infrequently met with. One of the former is to be seen in Fig. 169, C; and a very irregular form is shown in Fig. 170, D (× 200) in which the outer envelope is excessively thin, and the central protoplasmic mass has a very elongated shape.

The mode in which this resting phase originates from the ordinary spheres is shown in Pl. xvi., fig. 170 A, B (× 250). A large spherical body first appears in the midst of the brownish granular contents, as shown in A; the substance of the spherical body then contracts and separates from what becomes the inner envelope, which soon assumes a characteristic shape. The contracted central body is at first a mass of large fatty looking granules, and soon becomes surrounded by a definite limiting membrane. It gradually assumes the more homogeneous glistening appearance shown in Fig. 169, C, though remaining granulated at the periphery. This final change commences near the centre, and often gives the appearance of a large nuclear body such as may be seen in one of the two large specimens shown in Fig. 170, B.

As a rule, within the outer envelope, between it and the inner envelope, nothing but more or less of refuse, unassimilated pigmentary matter is to be found, but occasionally I have seen a curious admixture of the two changes which these Amœboid spheres are prone to undergo. That is, I have found in this space, mixed with the refuse matter, a number of large Amœbæ, though the thick outer envelope was still entire, as in Pl. xvii., fig. 171, A (× 500). I saw many such specimens on one occasion in old cells of *N. translucens*, but this happens to be the only photograph of the kind which I have preserved. I had attempted to stain the body with a dilute solution of thionin but obtained no good results therefrom.

This resting phase is met with at times much more abundantly than at others. Not infrequently I have seen nearly all

[1] More complicated excrescences of a very similar nature are shown around a resting Actinophrys in Pl. xvii., fig. 177, D. I have also seen shorter and very similar processes around large vesicles of *Schizochlamys gelatinosa* not unlike those represented by Cooke (" Fresh Water Algæ," vol. i., Pl. xxv., 2) in *Volvox minor*.

the spheres in a cell go through this phase of development, while in contiguous cells they have gone through the more common process which terminates with a speedy segmentation into Monads or Amœbæ. At other times, within the same cell, spheres promiscuously intermixed will be found undergoing side by side, and therefore under precisely similar external conditions, these different developmental phases. Sometimes, when thus intermixed, I have found the resting phase occurring more abundantly, or only, in the proximal regions of the cell, while in its distal half or two thirds the spheres have all been going through the common changes.

This same kind of admixture of two similar phases of a closely allied organism is also to be met with in Vaucheria, as may be seen in Pl. xvii., fig. 180. Up to the time of cessation of growth it is impossible to say which of the two changes any given sphere will go through, and as the two phases occur so often side by side it seems clear that the subsequent fate, that is, the appearance of the resting stage or the reverse, must depend upon some internal molecular change of an isomeric order. It is impossible that it can be due to external changes, looking to the way in which the two forms are intermixed within the same cell or the same filament.

It is in reality a veritable 'resting' phase, for these bodies remain for very long periods without undergoing any further appreciable changes. I was quite familiar with them over thirty years ago and they then puzzled me much, as I could discover no clue as to what further developmental changes they were likely to go through. And even now, during all the subsequent work I have done with Nitella, I should be ignorant of their ultimate fate had I not been able to follow rather more fully that of allied forms occurring in Vaucheria and elsewhere. Observation of these other examples has taught me that after more or less prolonged periods, the protoplasm in these resting phases breaks up into minute Monads or zoospores.

Up to the present, however, on only one single occasion have I come across evidence of such a change occurring in the resting phase of the Nitella organisms. This was in February, 1899, when, in a very old dead filament of *N. flexilis* covered with brown surface markings I found, among a number of very old spheres in the resting stage, four or five like Pl. xvi., fig. 170, c ($\times$ 375) in which the envelope that usually encloses the central mass of protoplasm was found much dilated and empty; while in another specimen, contiguous thereto, there were appearances indicative of a segmentation of the protoplasmic mass contained within this central envelope. There can be little doubt, therefore, that after long periods, as in the organisms found in Vaucheria and elsewhere, this central protoplasm is prone to segment into minute Monads or zoospores.

I have not yet been able to determine at will, with certainty, the production of this resting phase. Still I have often found bodies of this kind occurring plentifully in old cells that have been kept long in confinement. They are still more frequently to be met with when such cells have been shut up for a few days in dark pots with temperatures below 60° F; and, under similar conditions, I have recently found that even cells fresh from the ponds will yield a large percentage of these resting spores.

They are scarcely ever to be found when portions of the plant are placed under the most favourable conditions for the bringing about of rapid changes, such as occur when sprigs of either of the three species are placed in equal parts of tap and distilled water in a small closed pot and kept at a temperature of about 72°. When sprigs of plants fresh from the ponds are treated in this manner, the common changes are most rapidly and most thoroughly brought about. The digestive powers of the Amœboid spheres are then quite astonishing, seeing that a mass of chlorophyll corpuscles may often within two days be converted into colourless protoplasm which segments into Monads or Amœbæ—leaving either no remainders or only a very small amount of unassimilated refuse matter.

If the temperature is raised very appreciably above this—to 94° for instance—I have found that the change will still go on, though not so well, in sprigs of *N. translucens*. But with *N. opaca*, as I have several times found, at this temperature the ordinary changes no longer occur; the protoplasm of the cells seems to be speedily killed, and it subsequently undergoes none of the ordinary changes. This death of the protoplasm as a whole takes place occasionally from causes not easily recognizable, and in all such cases similar appearances are presented. I can now generally recognize this condition even with a good hand lens. The cells are darker, less translucent, and irregularities may be seen here and there in the chlorophyll layer. Examined with the microscope, the chlorophyll corpuscles often appear slightly shrunken, and they are as it were imbedded in a thin layer of granular and apparently coagulated protoplasm. When such changes as this are seen in Nitella cells one may be certain that no formation of Amœboid spheres will ever take place therein.

It happens not unfrequently that abortive specimens of the resting phase are met with, in which the central protoplasm degenerates and breaks up into a number of fatty-looking globules. This occurs especially in old plants long kept in confinement which have gradually lapsed into a less vigorous state. Under similar conditions also the development of the ordinary spheres, after they have attained their full size, becomes in great part arrested. The process of digestion and assimilation

is almost completely stopped, so that no peripheral colourless protoplasm is formed, though in the course of some days there appear, in the midst of the brown granular refuse matter, one, two, or sometimes three very sluggish specimens of Actinophrys, each of them surrounded by a granule-free space across which three or four pseudopodia may be seen to stretch. Such spheres are shown in Pl. xvii., fig. 171, B (× 375). They were contained within an old cell of *N. translucens*.[1]

On other occasions the peripheral colourless protoplasm may form and segmentation may commence, but, instead of being completed, this peripheral protoplasm becomes resolved into a mass of motionless Bacteria as in C (× 700)—a specimen which was found within an old cell of *N. flexilis*. As the Bacteria were motionless the photograph was taken without the aid of any reagent. The fact of their being motionless in the midst of a semi-solid medium, and uniformly distributed rather than aggregated here and there, is of course much more compatible with transformation than with infection.

The organism whose origin and developmental phases I have been endeavouring to trace has apparently been seen by Cienkowski only in its resting phase; as in his memoir entitled "Beiträge zur Kenntniss der Monaden"[2] he gives three small figures connected with this phase of the organism—and the organism itself he names *Pseudospora nitellarum*. This genus Pseudospora was included by de Barry[3] among what he termed "doubtful Mycetozoa"—along with Vampyrella, Colpodella and other genera. De Barry, however, admits, in reference both to Colpodella and Pseudospora, that these "two genera, as far as is at present known, do not form plasmodia." In the absence of this characteristic, which I can confirm, so far as Pseudospora is concerned, there would seem no reason why it should be classed even as one of the "doubtful Mycetozoa." In all the myriads of Monads and Amœbæ resulting from the segmentation of the common phase of this organism which have been examined by me within Nitella cells, I have never once seen any fusion of them. And only on two occasions have I ever seen fusion of two of the parent Amœbæ occur, when they have chanced to come into contact during their period of active growth. These organisms would seem to belong to the *Zoosporidia* of Zopf, and what has now been made out concerning their origin should stimulate investigations into the possible heterogenetic origin of other allied forms.

[1] The rays are of course not seen, as the application of a weak formalin solution to stop movements leads to their retraction.

[2] *Archiv. für Mikros. Anat.*, Band i., 1865, p. 203, Taf. xii., f. 11, 12, 13.

[3] "Fungi, Mycetozoa and Bacteria," Transln, 1887, p. 446.

(*e*) On two other Associated Changes seen within Nitella Cells.

I have long been familiar with a difference in the rapidity of growth of plants of Vallisneria in West Middlesex water and in the New River water respectively. They have always seemed to grow much more rapidly and vigorously in the latter than in the former, a fact doubtless due to some differences in the saline constituents of the waters, and the greater abundance of lime salts in that from the New River. When tap water has been mentioned hitherto it has always been West Middlesex water that has been referred to. I wished, however, to ascertain what would be the result of keeping Nitella for a time in New River water, and accordingly, towards the end of 1898, I made the following experiments and observations.

Fresh specimens of *N. flexilis* were kept for four or five weeks in an open vase on the mantelpiece containing this New River water, and portions were cut off from time to time and placed in small covered earthenware pots containing distilled water only, and allowed to remain there for a variable number of days.

On several occasions, after only three or four days of this change of conditions, I have found many of the previously healthy cells completely altered, and that often in quite different ways in contiguous cells. In each case, however, the cells had completely lost their bright green tint and had assumed a spotted or more uniform drab or earthy colour.

Two of these different changes in contiguous cells are shown in Pl. xvi., fig. 163, B (× 25). The terminal cell and the small bud on the left hand contained ordinary Amœboid spheres such as I have already described. While the cell with which they are connected and the contiguous cell (each of them shown only in part) were decolourised and crammed from end to end with a continuous layer of minute motionless Amœbæ which was found to have completely replaced the layer of chlorophyll corpuscles. This extraordinary transformation is shown in Pl. xvii., fig. 172, B (× 375) as it occurred in a terminal axis cell which was in a healthy condition when placed in the pot three days previously. Many of these minute Amœbæ contained brownish or olive coloured residual granules. Between them a few motionless Bacteria were to be seen here and there, but not a single green chlorophyll corpuscle was left.

This particular change has not been confined to specimens of *N. flexilis* treated in the manner above stated. I had not unfrequently met with it before I began to make use of the New River water, as my notes show, and always in association with the common change—that is, the two changes often occurred in contiguous cells in the most puzzling manner. I can find no details as to the conditions under which this particular change

was encountered, except in regard to one instance in which it also showed itself in a terminal axis cell. Fortunately in this particular case an early stage of the change was met with, in which the chlorophyll corpuscles were seen to be in different stages of decolourisation. It was found in a specimen of *N. flexilis* that had been in the house for some time, and had been in a dark cupboard during the last two weeks. The cell, part of which is shown in Fig. 172, A (× 375), had still a faintly green tint, but numbers of its chlorophyll corpuscles were either actually converted, or in the course of being decolourised and converted, as I believe, into such Amœbæ as are shown in Fig. 172, B. There can be little doubt that this remarkable transformation has been thus brought about.

In the cell of which B is a part the whole change had been brought about within three days—that is a healthy layer of chlorophyll corpuscles had been completely replaced by a layer of minute, motionless Amœbæ. In A, chlorophyll corpuscles of almost exactly the same size are seen undergoing the process of decolourisation; between the chlorophyll corpuscles, as between the Amœbæ, there are the customary Bacteria; and, in each case, in the early and in the perfected stage, we had to do with a terminal axis cell. And, in reference to this kind of transformation occurring in the specimens which had been in New River water, I find in my note book the entry,—"It seems especially prone to occur in the central buds at the termination of an axis."

It appears to me that these facts are only capable of being explained by such an interpretation as I have given to them. A healthy Nitella cell placed in distilled water is found in the space of three days to have all its chlorophyll corpuscles replaced by a layer of similarly disposed, motionless Amœbæ of like size. If we have not to do with a case of transformation, what has become of the chlorophyll corpuscles? Whence have come the minute motionless Amœbæ? And how have they been able to arrange themselves into such a uniform layer?

Soon after making these observations all the *N. flexilis* disappeared from the two or three habitats where I had previously been obtaining it; and until recently I have been unable to find specimens of this plant again, so that I have only been able to make a few additional observations on this subject. One of these was specially interesting. After examining a small terminal cell one evening in which the hyaline spheres were becoming granular and cyclosis was still active I put it alone in a covered watch glass with some distilled water, with a view to examining it again at a later stage. When I did examine it again, after only eleven and a half hours, I found that cyclosis had stopped, so I cut the cell

in a drop of eosine solution, and on examining the expressed contents I found multitudes of minute Amœbæ, and mixed with them slightly enlarged chlorophyll corpuscles which were becoming Amœboid. The corpuscles seemed to enlarge slightly and to contain only an admixture of green and colourless granules and then to assume an Amœboid activity. The different stages were seen with great distinctness, and more plainly than I have ever seen except on one occasion many years ago.[1] I took four photographs, but they were unfortunately all failures owing to the objects slipping during the process.[2] I was much disappointed because of the extreme distinctness of the different stages of the transformation.

It would seem that in some cases the change in the chlorophyll corpuscles must be almost universal, as in Pl. xvii., fig. 172, B, and as a consequence the Amœbæ remain of very minute size, owing to lack of food. But I have observed again recently on several occasions that the densely packed Amœbæ may be distinctly larger as in Fig. 172a, A, B (× 375). In such cases it may be assumed that more of the chlorophyll corpuscles remain unconverted and serve as food for the young Amœbæ, which consequently attain altogether larger dimensions. The inequality in size is very marked in B, and the peripheral protoplasm of many of the Amœbæ there may be seen to be segmenting into nucleated products in the usual way.

In all the cases where this kind of change is taking place I have observed again, as on previous occasions, that there is a more or less thick layer of motionless Bacteria outside and between the Amœbæ, and, of course, on the inner side of the wall of the cell, while the surface of the wall is thickly flecked with a number of curious glistening white specks, such as may be seen in C (× 375). A little above the proper focus these specks look black instead of white. Another interesting point is shown by this specimen. It was taken from one of the larger subterminal cells, in which the chlorophyll corpuscles were larger than they are in the minute terminal cells, and many of them also contained rather large starch grains. Several of such grains may be seen still undigested within the Amœbæ into which the chlorophyll corpuscles have been converted, after becoming as is usual slightly enlarged in size. In the next section I shall have to describe (p. 271) much the same kind of changes occurring

[1] "The Beginnings of Life," vol. ii., p. 409, fig. 81.

[2] This is particularly prone to occur and has caused many failures when attempting to photograph one or other of the expressed contents of the Nitella cell. The presence of this cell prevents the cover glass coming down sufficiently on minute objects, and slow sliding movements are consequently apt to be taking place even when the minute objcts appear to be at rest

in the chlorophyll corpuscles of *N. opaca* leading to their transformation into specimens of Actinophrys.[1]

In other portions of this stock of *N. flexilis* which had been kept in New River water, and subsequently transferred for a few days to a small closed pot containing distilled water, I found that the spheres which formed in the cells were notably different from those previously described. They seemed to be mere aggregates of dark olive brown globules (altered chlorophyll corpuscles) showing no visible envelope and, apart from colour, having much the appearance under the microscope of minute blackberries.

The majority of them, after a time, broke up and each gave exit to a single spherical or ovoidal body such as may be seen protruding from some of the spheres shown in fig. 173, A (× 375). The extruded bodies, liberated by the disintegration of the spheres, are shown in B (× 375), varying much in size and shape. They were finely granular bodies, showing in their substance one or two small circles having granular outlines. I made various attempts to procure some development of these bodies but never with any success. Some of them were kept in a damp chamber for fifteen days, without obvious change, except that they showed fewer granules than the fresh specimens.

Owing to the absence of any apparent bounding membrane and the ready disintegration of these spheres, heaps of the contained bodies of various sizes were often found massed together, as in C (× 150). One can only suppose that these bodies may be homologous with the central bodies in the resting phase of the ordinary spheres.[2] In these ordinary spheres the bounding membrane becomes notably thickened so that the contained body is rarely liberated; while in the blackberry-like spheres no boundary membrane is appreciable, and speedy disintegration is the result.

As to the mode of origin of this new kind of sphere I have been unable as yet to come to any positive conclusion. This remains as doubtful as the ultimate fate of the bodies produced in their interior, though the resemblance of the latter to what is seen in Pl. xvi., fig. 170, A, seems to leave little doubt as to their nature.

In some of the Nitella cells from this New River water the more common changes were, however, frequently met with. The brown globules (altered chlorophyll corpuscles) would in that case lose their individuality, at least in the peripheral portions

[1] A reference to the Index will show that chlorophyll corpuscles from other sources have been seen to undergo several quite different transformations.

[2] They will be seen, however, to have a closer resemblance to the resting-phase of the form found in Vaucheria (Fig. 180).

of the spheres, and would soon become digested and assimilated, leading to the formation of the usual translucent protoplasm. This was followed by its speedy segmentation into Monads or Amœbæ—though these products were usually distinctly larger than those commonly met with.

In other cases, where the conditions were less favourable, the contents of the spheres became resolved into a rather fine, pale brown, granular matter, in the midst of which one to three specimens of Actinophrys would show themselves in granule-free spaces, as in Pl. xvii., fig. 171, B.

The changes undergone by the Nitella in the New River water were, therefore, partly new and partly of well known types.

(*f*) On the Origin and Development of Swarms of Actinophrys within Nitella Cells.

Towards the close of the last section I referred to the development of one to three specimens of Actinophrys within spheres which had failed to undergo the usual stages of development. Under other conditions, that is, in previously healthy cells dying soon after they have been brought from the ponds, organisms of this type may also be met with, more or less sparsely distributed, in association with small Amœbæ, within the cells of *N. flexilis* and *N. translucens*. The two organisms are commonly of just the same size; they contain from 4-8 chlorophyll corpuscles, one or two only of which may be seen to be of a reddish brown colour while the rest have remained green. Such bodies, with or without rays (Actinophrys or Amœbæ), are commonly met with either in association with, or anterior to, the presence of certain small Ciliates which are also frequently to be found in large numbers within Nitella cells.

I am not now about to say anything further concerning either of these modes of appearance of Actinophrys, but rather as to their appearance in an altogether different fashion, and in prodigious numbers, *pari passu* with the disappearance of the chlorophyll corpuscles of the cell.

This change has occurred under different conditions, but I will first refer to a set of observations which I have repeated on four occasions with very similar results.

About thirty bright green terminal sprigs from a stock of *N. opaca*, which had been in the house for some weeks, were placed in a covered, glazed metal basin containing equal parts of tap and distilled water. During the remainder of the experiment the basin and its contents were kept at a temperature of about 60° F. At the end of a week on removing the cover for a mere momentary inspection, very many of the cells were seen to have become decolourised, and to be of a pale earthy tint. Five days

later almost all the cells had become decolourised in the same way—only one here and there still remaining green.

Microscopical examination of several of the cells showed them to contain multitudes of Actinophrys, many being absolutely filled with them. In most of the youngest terminal cells (those probably which possessed the highest vitality) there was the production of the ordinary Amœboid spheres, going through their usual changes; though one or more cells in many of the groups were found to present none of these organisms, and to be crowded with specimens of Actinophrys only. All the subterminal and other larger cells were found to be in a similar condition and crammed with Actinophrys, the only other organisms met with in these cells being dense masses of motionless Bacteria —an association which I find to be invariable in this kind of change. It is one which does not take place simultaneously throughout the whole length of the cells, but extends steadily from one end of the cell to the other. Roughly speaking the order is this: the Bacteria multiply more or less notably, while the green chlorophyll corpuscles disappear, and are replaced by specimens of Actinophrys.

Where this change is advancing through a cell careful examination will always show a mixed zone intervening between the healthy and the changed regions of the cell—that is between its green and its drab areas. In the latter region nothing but a dense mass of Actinophrys mixed with Bacteria and starch granules is to be seen—some of the specimens of Actinophrys being completely decolourised, while others show a few green mixed with colourless granules in their interior. Next to this area of complete change we have an intermediate zone in which small specimens of Actinophrys and chloropyll corpuscles are intimately intermixed; while beyond it we have green chlorophyll corpuscles only — all of them more or less loaded with starch granules or scales. This particular change being most prone to occur in old specimens of Nitella which have been kept for some time in the house and then treated as I have described, it will commonly be found that the chlorophyll corpuscles are, in such specimens, crowded with starch granules and are, as a consequence, very greatly enlarged.

A more thorough and searching examination of the intermediate zone will show that all the smallest specimens of Actinophrys are of just the same size as the chlorophyll corpuscles—that *none are to be found smaller than these corpuscles*; that they have at first no rays; that they are motionless; and that not one of them can ever be seen to divide.

What then is the mode of origin of all these myriads of Actinophrys which, within a few days, will appear within the closed cells of the Nitella? Do they come from without? This view

is negatived by the fact that none are ever to be seen making their way through the thick walls of the Nitella cells, though looking to their large minimum size, to their prodigious numbers, and to the fact that they are never seen to divide, thousands of them ought to be capable of being seen boring their way through the walls of the cells had they come from without. Further, these organisms are always at first completely motionless, and yet first show themselves intimately intermixed among the chlorophyll corpuscles.

It comes to this then. Myriads of chlorophyll corpuscles at first exist, and these become replaced by myriads of motionless specimens of Actinoprys, first showing themselves as bodies of about the same size as the chlorophyll corpuscles—a trifle larger but never smaller. How do the chlorophyll corpuscles vanish? And whence come the specimens of Actinophrys? These are the two related problems, and the only answer is, that the myriads of chlorophyll corpuscles are converted into the myriads of Actinophrys—just as, under other conditions, and in young Nitella cells whose chlorophyll corpuscles contain no starch grains, they may be simultaneously converted into minute Amœbæ, such as I have already described, and represented in Pl. xvii., fig. 172.

The mode in which the transformation of the chlorophyll corpuscles into the Actinophrys is brought about seems to be this. The corpuscle appears slightly to enlarge, and at the same time begins to decolourise, leading to the production of colourless mixed with fine green granules. The starch grains also begin to be digested. We have here the first stage of the motionless Actinophrys; spherical and as yet showing no rays. No entire chlorophyll corpuscles are ever to be seen within these specimens of Actinophrys, only at first a few green granules.[1] These coloured granules soon disappear as the organisms slightly increase in size, and where they are not too densely packed they may be seen to emit 6—8 pseudopodia, whose length about equals the diameter of the Actinophrys. Some of the chlorophyll corpuscles, failing to undergo this transformation, become disintegrated and liberate their starch grains, and such scanty food is devoured by those of the new organisms which come into contact therewith. The paucity of food, however, in comparison with the myriads of organisms, accounts for the fact that the organisms grow very little, and show only comparatively slight differences in size, as the figures will show.

I have seen this kind of change occur in recently cut cells as

[1] It cannot be, therefore, that the chlorophyll corpuscles are swallowed by the Actinophrys. The latter is of the same size as the chlorophyll corpuscle when, in its rudimentary form, it first shows itself among them.

well as in entire cells. And when it occurs in cut cells, it sometimes gradually spreads from the open extremity inwards; but just as frequently I have seen it begin at the inner and closed extremity of a cut cell and gradually spread outwards.

Some illustrations will now help the reader more fully to realize what I have been describing; but, as photographs can only show the crude results rather than the minute details of this process, it seemed best to reserve them till the description of what occurs had been given.

In Fig. 174 (× 150), a portion of a cell of *N. opaca* crammed with recently formed Actinophrys, together with partially decolourised chlorophyll corpuscles and masses of motionless Bacteria, is shown. Many of the specimens of Actinophrys still contained green granules in their interior, though others were completely decolourised.

I have already noted the fact that in *N. opaca* the chlorophyll corpuscles are always larger than in either of the other two species (p. 245), and when they are filled with large starch scales they undergo a further notable enlargement, as was the case in the cell from which Fig. 175, A (× 375) was taken. This figure shows an intimate mixture of enlarged chlorophyll corpuscles packed with starch scales, and embryo specimens of Actinophrys, partly decolourised, into which others of the corpuscles have become converted. All stages between the two were to be seen in this specimen. They were all motionless and the photograph was taken without the use of any lethal reagent. All that was to be seen on the surface was an accumulation of Bacteria and Leptothrix.[1] In B (× 200) a portion of a completely decolourised cell of *N. translucens* is shown crammed with colourless specimens of Actinophrys. Being more developed, these organisms are liable to exhibit slight though scarcely perceptible movements, so that a weak solution of formalin was used before the photograph was taken. Bearing in mind the small size of the chlorophyll corpuscles in *N. translucens* it will be noted that, where they are small, the specimens of Actinophrys are similarly small. A comparison of Fig. 175, A and B will make this plain, even when due allowance has been made for their different degrees of enlargement. The same greater size of the specimens of Actinophrys, with which another cell of *N. opaca* was densely packed, is shown in Fig. 176, A (× 125). The organisms here were all completely decolourised, and, through the centre of the cell, a large dense aggregation of Bacteria was to be seen, in the midst of which many of the Actinophrys were embedded.

[1] The surface of the cells represented in Figs. 174, 175 B, and 176 A were even almost free from such organisms, and showed not the least indication of Amœbæ or Actinophrys penetrating from without

I have tried on several occasions to get photographs of these specimens of Actinophrys, but with no success. All reagents have proved useless because they at once entail a retraction of the pseudopodia. However motionless the Actinophrys appears to be, the photograph generally shows that it is only relative. The best I have been able to do is shown in Fig. 177, A (× 375), which was taken with a very rapid plate and an exposure of one minute. The nucleus and nucleolus are dimly to be seen together with six or more rays; and this represents the common appearance of the organism in its active state. After from about seven to ten days, when their scanty food is more or less exhausted, this active state is over and the organisms then encyst themselves, when they present the appearance shown in Fig. 176, B (× 375).

Once in the encysted condition specimens of Actinophrys seem to remain long without undergoing any further change. I kept some of the above described specimens in a small tube for about six weeks, but at the expiration of that time they showed no appreciable alteration, except that the enclosed protoplasm seemed a little less granular than it had been previously.

I have already alluded to the fact that the Monad, the Amœba, and the Actinophrys are only different phases easily convertible the one into the other. This is thoroughly recognised as regards the Monad and the Amœba, and fresh evidence thereof is constantly to be met with in studying the results of segmentation of the common Amœboid spheres in Nitella—sometimes the products appear as Monads and sometimes as Amœbæ, without any reason for this variation being apparent. During my examination of the specimens of Actinophrys crowding the cells in *N. opaca*, wishing to preserve one of the cells mounted in water for future observation, I smeared round the edge of the cover glass with vaseline so as to prevent evaporation, and on examination after an interval of twenty-four hours, all the specimens of Actinophrys were found to be converted, in this confined situation, into typical though rather sluggish Amœbæ, some of which are shown in Fig. 177, B (× 375).

In cells of a specimen of *N. translucens* which had been in a dark cupboard for ten days I, on several occasions, found some very large specimens of Actinophrys gorging themselves with chorophyll corpuscles, and also containing one to four finely granular spherical masses. One of them, when placed in a drop of a one-sixth per cent. solution of osmic acid, at once burst, and became resolved into fine granules. Another was treated by drawing a drop of the solution under the cover glass, and the result was the deformed specimen represented in Fig. 177, C (× 375), with rays shrunk or altogether retracted. Other of these specimens seemed to lapse into a resting stage, without

becoming actually encysted, and then there was developed from them a close fringe of motionless excrescences of some kind, generally in a single row; though in a few specimens the excrescences were extremely dense and branched as in D (× 450), the photograph of which was taken without the use of any reagent. I have never, before or since, met with specimens exactly like this, though excrescences of much the same kind are shown in Pl. xvi., fig. 169, C.

I have shown hitherto that Amœboid organisms arise in two distinct modes in Nitella cells; (*a*) from transformed hyaline spheres, and (*b*) from transformed chlorophyll corpuscles, when they appear either as Amœbæ or as Actinophrys. There is reason to believe, however, that they also arise in a third way, namely (*c*) by growth from microscopic particles developing in the semi-fluid protoplasm, as indicated in Fig. 184, and after the same manner as that in which they will be found to make their appearance within Vaucheria filaments. It seems possible that they take origin in this manner in *N. translucens*, in which hyaline spheres are almost completely absent. I have, however, as yet not attempted to investigate this subject.

Remarkable as are these changes occurring within dead and dying Nitella cells, they are only a little, if at all, more so than those normally occurring within healthy Nitella cells. There is the origin within their protoplasm of thousands of chlorophyll corpuscles, themselves multiplying by fission; the origin of hundreds of hyaline spheres; of hundreds of the mysterious plastides, also multiplying by fission; and almost as many of the equally mysterious bur-like bodies enclosed within their delicate hyaline envelopes; and lastly there is the process of cyclosis itself. When one thinks of all this occurring within cells in which no nuclei exist the marvel of it is still further increased. I have certainly never been able to detect a nucleus within the developed Nitella cells, while Sachs, speaking of the commencement of cyclosis definitely says that the nucleus about this time becomes absorbed.

I have made many observations on the appearance of Ciliates of different kinds within the cells of Nitella, but they are scarcely complete enough to be dealt with now. And much the same thing may be said in reference to the origin of Rhizidia and other allied Fungi which are often very abundant within Nitella cells. I shall pass on, therefore, to describe some very common changes occurring in Vaucheria filaments. Before doing so, however, I should like to call attention to some peculiar growths or depositions commonly to be met with on Algæ and other aquatic plants. Some of these are shown as they occur on old Nitella cells

(Fig. 178, A, × 50); on old thalli of Duckweed (B, × 375); and also on old Vaucheria filaments (C, × 250). As to the nature of these growths (for they seem really more like growths than mere depositions) I am quite in the dark, having been unable to obtain any information concerning them. There is a sort of common plan about them all. They are of a dark brown colour; they almost always show a central aperture or space, and sometimes around this aperture, as in the very unusually large specimens shown on the Vaucheria filament, there is a thick, raised, collar-like rim. Brown markings, seemingly very similar, are often found around the point of attachment of small epiphytes on Algæ, or on the thalli and leaves of Duckweed and other aquatic plants.

XX. ON TWO HETEROGENETIC CHANGES OCCURRING WITHIN VAUCHERIA FILAMENTS.

(a) On the Production of Amœboid Organisms allied to those commonly found within Nitella Cells.

The first of the two changes now to be referred to is very similar to that first described as occurring in Nitella—leading to the production of Amœboid organisms to which the generic name Pseudospora was given by Cienkowski.

This change has only been met with when the weed has been exposed to one or other set of unnatural conditions, and where its death as an Alga has been brought about slowly.

I have made no observations showing the rapidity with which the changes in question occur in Vaucheria, similar to those recorded for the development of the organisms forming in Nitella cells, but my impression is that they take place much more slowly—especially in the later stages leading to segmentation into Monads, these being stages which I have only seen on comparatively few occasions,

Most of the conditions under which these changes have occurred will be indicated in referring to the different figures.

In Fig. 179, A (× 300) a number of Amœbæ in different stages of development are shown in a small Vaucheria filament taken from a stoppered bottle, into which portions of a healthy plant were placed three months previously. The bottle was filled with tap water, and thereafter left on a shelf in a dim light. The Amœbæ, though varying in size, are mostly small; the chlorophyll corpuscles on which they have fed having been partly digested and converted, from the periphery inwards, into colourless protoplasm. In B (× 375) the largest Amœbæ of this kind I have ever met with in Vaucheria are shown. They were contained in filaments that had developed from Zoospores, which

were subsequently kept in the house, exposed to ordinary daylight for three months, in a shallow glass covered by a small inverted beaker. Then during another month, while I was away on a holiday, they were left in a dark cupboard.

On my return I found many of the filaments still green, though the chlorophyll in some of them was much disarranged and had a roughly spiral disposition. Other filaments were decolourised, and these, on examination, were found to be crowded with Amœbæ in their ordinary or resting phases, irregularly intermixed. In some of the filaments one or other of these forms preponderated; while in others the two phases were more equally intermixed, as may be seen in Fig. 180 (× 375) showing portions of two contiguous filaments. Several of the spheres showed indications of being about to segment at the periphery into Monads, while segmentation was actually seen in others—the resulting Monads being small, about $\frac{1}{4000}$ of an inch in diameter. Some of them were spherical with a single flagellum; while others were more oat-shaped or spindle-shaped—variations in form that were seen also among the Monads derived from the Nitella Pseudospora. Young Amœbæ, as products of the segmentation have, however, never been seen in Vaucheria.

The resting phase is generally met with in Vaucheria very much more frequently than the ordinary form. It is also a simpler body than that found in Nitella; seeing that the included sphere of protoplasm is contained within a single rather than within double envelopes. The mode in which this resting stage is developed was clearly observed in one filament where they were forming. What appeared to be a mere round or oval excentric space was first observed. This was really, however, a mass of hyaline protoplasm, which gradually developed a definite wall or limiting membrane, as the mass itself became more distinct and more granular in appearance.

Outside this central body there is a mere aggregate of refuse granules mostly contained within a thin envelope. This latter, after a time, is apt to give way, so that the granules become dispersed; in others, however, the outer envelope is much firmer and may persist for two, three or more months. In some specimens, as in the corresponding forms found in Nitella, the central mass of protoplasm is prone to degenerate, and becomes resolved into a number of rather large, fatty-looking globules.

There is one point of great significance to be observed in reference to the production of the resting phase. It is shown plainly in Fig. 180 that this form occurs not only in contiguous filaments, but even side by side with the ordinary forms within the same filament. External conditions are, therefore, similar for both, so that we are compelled to conclude here, as we were in regard to the production of the resting stage in the Nitella

organism, that its production must be due to some internal molecular changes, as a result of which the assumption of the resting phase becomes a necessity.

The ultimate development of this resting phase I have now seen on many occasions in Vaucheria filaments which have been shut up in water for three or four months within closed stone pots. In four or five weeks, or less, filaments exposed to these conditions will be found to be crowded with specimens of the resting phase. They have, however, to be kept for the longer period in order to bring about their development. We may then see such specimens as are shown in Pl. xvii., fig. 181, A (× 500) where the central mass of protoplasm has segmented into 10—12 Monads or zoospores, and in a larger specimen (B, × 500) in which many of the Monads have escaped, while others are to be seen still within the inner envelope or between it and the outer one. This last specimen shows too how the inner envelope has become stretched and dilated by the swarming movements of the Monads before they effect their escape. In old specimens of Vaucheria, taken from the closed pot, multitudes of specimens were found in this condition, as in C (× 375), in which some very large empty envelopes were mixed with smaller spheres in which no development had as yet taken place.

Sometimes the Amœboid spheres as they develop project rays and take on the forms of Actinophrys. These may often be seen of about equal size within the Zoospores of Vaucheria or young filaments developed therefrom. A portion of such a filament is shown in Fig. 182, B (× 250) which had been in a pot covered by an inverted glass for ten days. The filaments were mixed with Zoospores having similar contents. Some of the specimens of Actinophrys were dark green and gorged with chlorophyll corpuscles, while others were partially decolourised. In A (× 375) similar organisms were found crowding more mature specimens of Vaucheria which had been confined in a dark pot for two weeks. They were nearly all in an earlier stage of development and of a dark green colour. Unfortunately the rays in each case were, as usual, retracted under the influence of the weak formalin solution used to check their movements before the photographs could be taken.

I have no reason to think that these specimens of Actinophrys have any different mode of origin in Vaucheria from that of the ordinary Amœboid spheres. We know, of course, that these two organic forms are often interchangeable; and when Vaucheria dies in media, not cut off completely from light and fresh air, the Actinophrys is the form most commonly met with.

We now have to consider the important question as to the origin of these organisms which are to be found so abundantly in

Vaucheria filaments exposed to such conditions as I have mentioned. Do they come from without by some process of infection? Or are they produced from the substance of the protoplasm in the dying Vaucheria?

I know of no evidence in favour of the former hypothesis, and all the facts seem to point to the truth of the latter view. Thus Fig. 183, A (× 375) represents a small partially decolourised filament of Vaucheria taken after two weeks from a closed pot with a view to studying the early stages of change. The specimen was placed in a very weak solution of eosine and allowed to remain therein for twenty-four hours, as I had previously found that young Amœbæ would live for two or three hours in such a solution, and, after their death, would become stained of a rose colour.

On subsequent examination with the microscope this filament was found to be comparatively clear on the external surface. There was no army of Monads or Amœbæ attempting to bore through the wall of the filament, but within, its protoplasm contained multitudes of minute Amœbæ stained of a rose colour, and varying much in size, though the largest of them was scarcely more than $\frac{1}{4000}$ of an inch in diameter. These minute Amœbæ were intermixed with chlorophyll corpuscles, and with mere granules of different kinds, some of which may have been earlier stages of the minute Amœbæ. In B (× 600) another of these old Vaucheria filaments is shown, under a higher power, which had been similarly but more strongly stained with a weak solution of eosine. In this filament, together with young Amœbæ, there were seen amorphous masses of the Vaucheria protoplasm that had become stained of the same rose tint as the Amœbæ themselves. This latter fact, therefore, tends to support the view, to which all the other evidence points, viz., that the young Amœbæ, which soon begin to devour the chlorophyll corpuscles are only individualised portions of the vegetable protoplasm.

There are no elements here, like the hyaline spheres within the Nitella cells, from which the Amœbæ may have taken their origin. It would seem that in Vaucheria they develop within its protoplasm from scarcely visible particles, which remain motionless as they increase in size up to about $\frac{1}{4000}$ of an inch in diameter, when they begin to feed upon the chlorophyll corpuscles and rapidly increase in bulk. As a rule in Nitella cells undergoing the common change, as I have pointed out (p. 253), the small Amœbæ are not often to be seen much beneath this size. Still, in certain cases Amœbæ seem to take origin in Nitella protoplasm as I am supposing they do in Vaucheria, that is, from scarcely visible particles gradually increasing in size. In illustration of this I may point to Pl. xvii., fig. 184 (× 560), which was

taken from a cell of *N. flexilis* in which the blackberry-like spheres were developing. This cell had been left on a microscopic slip and beneath a cover glass, in a damp chamber for twenty hours. At the expiration of this time a large number of minute Amœboid bodies varying in size from mere particles upwards had shown themselves, such as may be seen in the figure.

In reference to the origin of the Amœbæ within the Vaucheria filaments the evidence is of this nature. Though the organisms exist in great numbers within the filaments, they are almost motionless, and are never seen to divide. If they do not divide, and if they really come from without, then each one of these almost motionless organisms must in some way have made its way through the wall of the filament. Again, the organisms found within are most frequently seen (as shown in Fig. 182) to be in just the same stage of development. This would imply that perforation from without, if it occurred, must have been effected by large numbers of them simultaneously—yet nothing of the kind can ever be seen.

(*b*) On the Origin of Pigment-bearing Amœbæ within Vaucheria Filaments.

In Vaucheria filaments which have been confined in the dark in a stone pot for seven to ten days or longer, as well as when they have been kept under other unhealthy conditions, though not so completely cut off from all light rays, it is common to find among the chlorophyll corpuscles single particles, or small aggregates of them, of a greenish black colour, such as are shown in Pl. xvii., fig. 185, A (× 375).[1] This specimen of Vaucheria had been shut up in a pot for two weeks.

Close examination of these aggregates of pigment grains shows that they are not at rest. They exhibit very slight oscillatory movements, which always have to be arrested by weak solutions of osmic acid or formalin before photographs can be taken. Sometimes these aggregates of granules are quite formless, as in B (× 375), but in all cases they seem to be held together by, or imbedded in, some invisible substance. In other cases, as in C (× 375), it can be seen that the pigment granules are contained within a very minute sphere of hyaline protoplasm, in which they are closely packed. And in other cases still, only two or three of these greenish-black granules are contained within a minute hyaline sphere or vesicle, and the granules are then seen to present slight movements therein. Such bodies slightly

[1] On one occasion I found that the particles had a reddish-brown or rust colour, otherwise they have always been of a greenish black tint, though just above the proper focus they look more like fat particles.

stained by a rather strong osmic acid solution are shown under a high magnification in D (× 700).

Single granules of this kind are not common; they are rarely in groups of less than 3—5, and though they exhibit slight oscillating movements, these aggregates are not obviously contained within a cell or envelope. Nor are free cells of any kind to be seen capable of swallowing such pigment granules.

The same obscurity which reigns concerning their origin obtains in regard to their growth. The aggregates of pigment granules go on increasing in size and become more or less spherical in shape as in E (× 500). The pigment seems to be produced within their substance as a product of metabolism. Certainly no loose pigment granules are to be seen around them; nor can the aggregates ever be observed to take any solid particles into their interior, after the fashion of the Pseudosporæ developing in Nitella or Vaucheria.

Such aggregates have been often met with abundantly in Vaucheria zoospores and in the filaments developing therefrom, as well as in filaments developed from Vaucheria resting spores, whenever they have been kept for a time under unhealthy conditions. In Pl. xviii., fig. 186, A (× 375) a number of these organisms are shown within a filament which had developed from a resting spore, varying in size but nearly all distinctly spherical. Some of the largest specimens of these organisms that I have yet seen showing no trace of peripheral clear protoplasm, are represented in B (× 375) closely adherent to the wall of the filament, and with a consequent alteration in shape giving the first indication of their Amœboid nature.

The later phases of these organisms have only been seen in filaments developed from resting spores of Vaucheria which had been kept for several months in a rather dim light and beneath a bell-jar. It will be seen moreover that the changes they have then undergone have been essentially similar to those met with in the pigment spheres which made their way out from the germinating resting spores, as described in pp. 196—202. There also we had to do with aggregations of pigment granules which, in some mysterious way, become included in protoplasm so minute in amount as to be at first invisible. Gradually, however, a digestion of some of the pigment seemed to occur, with the formation of a peripheral layer of brownish protoplasm, which subsequently underwent segmentation into Monads, as shown in Pl. xiv., fig. 140, A.

Here also, in these pigment-bearing Amœbæ formed within the Vaucheria filaments, we have, after a long time, the formation of a peripheral layer of whiter protoplasm (C, × 375), which ultimately begins to show signs of the ordinary segmentation into Monads as in D (× 375). Another specimen was found in which

the segmentation was more complete, but unfortunately the photograph I took of it was spoiled. In this specimen the outline of the Monads was distinct, and each was seen to possess a nucleus. What is seen in D corresponds with the stage of *P. nitellarium* shown in Pl. xvi., fig. 164, D.

Details concerning the earliest stages of these strange organisms can often be best made out at the extremities of filaments, where the chlorophyll corpuscles are often very sparsely distributed. Here separate and small groups of the greenish black particles, devoid of protoplasm, may be seen, together with other irregular groups imbedded in a small amount of hyaline protoplasm and presenting almost imperceptible movements. These seem to be the earliest stages of what subsequently become the pigment-bearing Amœbæ whose development I have just been describing. No cells or organisms, other than Bacteria, are usually to be seen in association with these early stages. But, occasionally, I have found in some filaments, with the early stages of these pigment Amœbæ, a few very minute spherical bodies like mere hyaline vesicles, containing a central dot or nucleus. One of these minute bodies is shown under a high magnification in Fig. 186, E (× 700). They have rarely more than about one-third of the bulk of the chlorophyll corpuscles among which they are found. They have never been seen to grow or to multiply; to vary in shape or to take anything into their interior; and I do not think they are in any way related to the pigment-bearing Amœbæ whose origin I have been endeavouring to trace.

It is clear from what has just been said that these pigment-bearing Amœbæ within Vaucheria filaments, as well as the remarkable organisms taking their origin from the pigmentary aggregates contained within Vaucheria resting spores, go through the same developmental phases as the common forms of Pseudospora that take their origin within Vaucheria filaments and Nitella cells. The latter are, however, most voracious organisms which go through most of their life phases very rapidly; while the former, loaded with comparatively indigestible pigment granules, have a long-drawn-out existence.

Then, again, allied organisms that apparently go through similar developmental changes, which I in earlier parts of this work found it convenient to refer to merely as 'Monad Cysts,' have been shown to originate from actual segments of other Vaucheria resting spores (Pl. ii., figs. 17-19); within encysted Euglenæ (Fig. 21); from the substance of the resting eggs of a Rotifer (Pl. iv., fig. 36); and also within the filament cells and resting spores of some varieties of Spirogyra (Pl. xiv., figs. 141, 147). In not one of these cases has there been the least evidence tending to show that the presence of the organisms in the

situations mentioned could be accounted for by any process of infection, nor have organisms of like kind ever been seen externally in association therewith.

XXI. ON DIFFERENT HETEROGENETIC CHANGES OCCURRING WITHIN SOME DIATOMS.

The specimens now to be briefly referred to were found among masses of intermixed Oscillatoria and Spirogyra floating on the surface of a pond. Among these matted weeds there were crowds of Navicula, Pleurosigma, Stauroneis and other Diatoms. After some of the masses of Algæ had been kept in the house, from seven to ten days, very many of the Diatoms were found containing microörganisms of different kinds, or with their nuclei greatly altered.

That the Diatoms were entire, and that their cavities were practically closed, was shown by the fact that, where they contained active Bacteria, the movements of these were only arrested after the Diatoms had been soaking in a quarter per cent. solution of formalin for an hour or more; while any such moving organisms outside were killed at once or had their movements immediately stopped by this same solution.

In the specimen from which Fig. 187, A (× 375) was taken seven or eight Amœbæ were seen among the massed green and decolourised endochrome of a Pleurosigma, which they were devouring; while, as shown in B (× 500), about eighteen of these organisms were seen in another specimen intermixed with brown and red pigmentary refuse granules.

In C (× 375) a Navicula is shown having its nucleus altered by small outgrowths therefrom and with rows of motionless Micrococci along the margins of the endochrome. Many others were seen in which the Micrococci were much more numerous mixed with minute threads like Leptothrix and with brownish yellow endochrome. The whole cavity of the Navicula in these cases was generally stained either of a yellowish or greenish colour, resulting from changes in the endochrome. Of these organisms no satisfactory photographs could be obtained.

Part of a large Stauroneis is shown in D (× 375) which contained in its interior a few Micrococci and many active Spirilla. The Diatom was entire and the organisms within were difficult to kill, but the specimen must have slipped slightly on the stage of the microscope so that the whole of it was not photographed. Other specimens like this were seen, and also a Stauroneis containing a number of Amœbæ surrounded by brown granules mixed with greenish endochrome, very similar to what is shown in A.

Multitudes of Pleurosigma were seen in which the nucleus had

undergone a remarkable change, though, in others, both nucleus and nucleolus were still quite distinct and unaltered, as in Fig. 188, A (× 375). In the cases where the nucleus was altered what appeared to be large outgrowths from it were seen, as in B and C (× 375). The change was so extreme in some specimens as to hide or render indistinguishable the original nucleus, as in D (× 375), though in E the original nucleus is seen with a number of outgrowths from its periphery. In another specimen a minute mycelium had developed from some part of such an outgrowth. Very fine threads were seen amongst it, and one of them had made its way out between the valves and was seen projecting therefrom.[1] In B, C and E little spheres are forming such as might give rise to mycelial threads; and in other specimens a number of Micrococci or motionless Bacilli were visible in the substance of the outgrowths.

In the case of these motionless Bacteria appearing in the substance of the nucleus, and no where else within the Diatom, as well as in the case of the motionless Micrococci which have been seen so frequently appearing in the midst of the endochrome, the notion that they are there owing to some process of infection from without is hard to believe. No means are known, again, by which Amœbæ can make their way into a Diatom. On the other hand, as I have previously shown, they may be formed from the endochrome of other Algoid cells, from resting spores of Spirogyra or Vaucheria, as well as from the substance of Euglenæ and the spheres contained within a Volvox (see p. 236).

These changes in the nucleus of specimens of Pleurosigma are interesting from the point of view of the discussions that have been taking place for some years in reference to rival views as to the nature of the so-called "cancer body." Many workers regard this body as, in reality, merely a modified cell nucleus; while others look upon it as an independent organism—a protozoan of some kind—and base upon its presence their belief in the parasitic nature or origin of cancerous growths. Seeing that heterogenetic changes are so common in lower organisms, it seems not improbable that some representatives of such processes should occur in the bodies of higher organisms, in sites where unnatural or morbid processes are actually taking place. Such organisms, for instance, as Sarcosporidia[2] and allied forms, may be capable of arising by heterogenesis within the cells of animals, just as positive evidence will be forthcoming concerning the origin of Bacteria in this manner.[3]

[1] This specimen was unfortunately moved slightly while a photograph was being taken, so that it is too indistinct for reproduction.

[2] See "Protozoa and Disease," by J. J. Clark, 1903, p. 102.

[3] Concerning the occurrence of such processes see pp. 307-312.

XXII. FURTHER OBSERVATIONS ON HETEROGENETIC TRANSFORMATIONS OCCURRING IN THE SUBSTANCE OF HYDATINA EGGS.

What I have to say here is to be regarded as a sequel to Section IX. (pp. 124-138), and I will first deal with the question of the ultimate fate of the encysted Otostomas, some statements concerning which were made at the close of that section.

My object then was to show that the encysted Otostomas were totally different from, and not easily capable of being confounded with, Otostomas that had just come into existence by a transformation of the substance of the Hydatina egg, and which could be seen, either stationary or revolving, within the unbroken egg-case. The thin egg-case is quite different from the thick walled cyst; and the embryo Ciliate within the egg-case is just as different in texture from the greatly enlarged Ciliate which, after a period of activity, encloses itself within a cyst—as I then showed.

That being my object I did not, at the time, mention the fact that on the day on which I placed portions of the pellicle, containing the Otostomas and other Ciliates, in some fresh tap water within a beaker that was left on the mantelpiece, I also placed a smaller quantity of this pellicle within another beaker containing tap water, covered it with a circle of glass, and then put the vessel into a dark cupboard.

The contents of this latter vessel were either not very carefully examined till six weeks had elapsed, or else I found nothing very different from what I was finding in the open vessel exposed to the light, as there are no entries in my note-book in regard to such examinations. As I have already stated (p. 137), at the expiration of one month all the Otostomas in the open vessel had become encysted; and so, in all probability, had those in the dark cupboard. But, after six weeks, it is noted that I found several of the encysted specimens in this latter vessel in the condition shown in Pl. xviii., fig. 189, B (× 250), in which the entire contents had become replaced by a vast number of resting Amœbæ, just such as may be seen in Pl. xvi., fig. 165, C and D. The distended cyst was unfortunately ruptured by the weight of the cover-glass. The small cyst, and portions of others that are to be seen, are those of small Vorticellæ.

Judging from other specimens found, showing earlier stages of this change, it seems clear that the minute Amœbæ, with which the Otostoma cysts were filled, were not produced therein, like those shown in Fig. 165, by the simultaneous segmentation of the substance of the encysted Ciliate, but were produced rather after the fashion that occurs in the large resting spores of a Spirogyra, such as may be seen in Pl. xiv., fig. 142, A, B. In some of the specimens the substance of the encysted Otostoma

had become resolved into a semi-fluid magma in which there were obscure indications of new units appearing; while in others motionless Amœboid corpuscles of different sizes were to be seen appearing discretely throughout the substance of this semi-fluid magma, as shown in the half of one of these cysts represented in Fig. 189, A. There was, moreover, no indication of the occurrence of multiplication by fission.

In the open vessel exposed to light the embryo Otostomas were found alive for over three weeks after this date; some of them showing active contractile vesicles, and slowly revolving within their cysts—especially after they had been exposed for a time to the light of the lamp.[1] But a few days later I found the first of many specimens undergoing similar changes to those shown in Fig. 189, and no more living Otostomas were subsequently met with. The conditions were evidently not favourable for their reappearance as Ciliates. It is interesting, however, to note how much more quickly they underwent retrograde changes when they were supplied with very little air and were cut off from light.

(*a*) Additional Remarks Concerning the Transformation of Hydatina Eggs into Ciliated Infusoria.

I had been accustomed to get my supplies of Hydatinæ for six months from a site where they were actually swarming; and it was their abundance which enabled me to make so many observations and experiments, during that period, upon the transformation of their eggs into Otostomas and other Ciliates. This site was then temporarily destroyed, as I have stated on p. 136, by work in connection with the laying of a main sewer; and, from that time to this (a period of eighteen months), I have met with an extraordinary dearth of Hydatinæ, and have been able to make no further observations on this subject. Although the ditch from which I obtained the Hydatinæ originally has been apparently restored to its former condition; though it still receives drainage from the farmyard, and though from time to time Euglenæ have been present in abundance on its surface; yet, on the various occasions on which I have taken away samples as before, I have been disappointed and have found no Hydatinæ. This is a provoking illustration of the fact mentioned in the quotation from Pritchard on p. 350; and, unfortu-

[1] The concentrated light from the lamp seemed decidedly harmful to them. One which I had been examining carefully as it was more active than the others, was, at the close of the examination, placed in a watch-glass with some fresh water in the hope of my being able to trace its further development; but, when I looked at it again three days later, it was found to be dead and no longer, as before, completely filling the cyst.

nately, I have not since, during exceptionally wet seasons, been able to find other good habitats. The few additional remarks that I have now to make are based, therefore, upon observations previously made before the old supplies were exhausted.

I then had an opportunity of making a few more observations upon the presence of Oxytrichæ and their matrices within the eggs of Hydatinæ. I have previously shown that the small, as well as the large eggs of this Rotifer, may become transformed into Otostomas (Pl. x., figs. 100 and 103); and that the large eggs may also be resolved into a number of matrices from which Vorticellæ develop (Pl. iv., fig. 42). On p. 44 I referred to observations, made more than thirty years ago, in which I saw eggs of Hydatinæ full of matrices, mixed with developed specimens of Oxytrichæ, within unbroken egg-cases of this Rotifer. Two years ago I again found indications of this change (referred to on p. 45); and similar indications were encountered in the spring of last year, when working with the last batch of Hydatina eggs with which experiments were made. In neither of these cases, however, were the egg envelopes quite full and unbroken, as they had been in the observations first made.

The last observations were of this nature. In a thin pellicle, about nine days old which had been in a dim light, I found, where it was in contact with the surface of the bowl, several small Hydatina egg-cases containing matrices and active Oxytrichæ. One of these specimens is shown in Fig. 190, C (× 200) in which were contained fifteen small matrices together with one active Oxytricha—the latter being hidden in the photograph. I found also three small Hydatina eggs close together, each containing a mixture of matrices, in some of which the Ciliates were revolving, together with free and active Oxytrichæ; but, whilst arranging to photograph them, I unfortunately pressed upon the cover-glass, and thus displaced and damaged the specimens. I could only photograph the best of the damaged specimens, therefore, as it is to be seen in D (× 250). It contained one active Oxytricha (on the left), and four matrices, though two of the latter, as shown, are much out of focus. In one of the large eggs also four active Oxytrichæ were found, one of which was undergoing fission, as may be seen in Fig. 190, B (× 250).

There is this point worthy of note. On each occasion that Oxytrichæ or Vorticellæ have been found within Hydatina egg-cases they have always been taken from thin pellicles on the surface of the fluid. On the other hand, when the eggs have been placed in pots—some of them remaining at the surface and some sinking—I have always found that their transformation into Otostomas has occurred most numerously among those groups of eggs that have been taken from the bottom of the pot.

The changes in the Hydatina eggs which lead on to the pro-

duction of the Oxytricha and Vorticella matrices are, I believe, such as are shown in Figs. 48, 49, and Fig. 51, A, in which the egg substance, near the mid-period of the transformation, becomes resolved into a number of large (though unequal) spherules, quite different from those that are produced in eggs which become converted into Otostomas (such as are shown in Figs. 99 and 100).

Other eggs are found, however, in which the contents become resolved into granular and hyaline spherules intermediate in size, such as I have shown in Fig. 190, A ($\times$ 250). It may subsequently be ascertained that a larger number of smaller Ciliates are produced from such eggs—either *Aspidisca costata* or other forms (see p. 53). It will be observed that the spheres in Fig. 190, A, as well as those shown in Figs. 48, C, D, and 51, A, are unequal in size; but so also are the matrices represented in Figs. 42, C, D, 50, A, and 190, C.

In reference to this whole question of the transformation of the eggs of Hydatina into Ciliated Infusoria three important points must never be lost sight of by those who attempt fairly to weigh the evidence adduced.

(1) The first and most important point is this, that *during the ordinary development of a Hydatina, the egg is never converted into a mass of spherules large or small, such as my photographs show* when, as I allege, they are being converted into Ciliated Infusoria.

(2) When these spherules are converted into 15 — 20 or more matrices, from which Oxytrichæ or Vorticellæ subsequently issue, and when this occurs within an unbroken egg envelope as shown in Fig. 42, C, nothing but transformation of the original substance of the egg could account for their presence. And, even in the case where the egg envelope is not entire, the presence of a number of closely packed matrices (as in Figs. 50, A, and Fig. 190, C) can only be reasonably accounted for on the supposition that they are results of transformation and have been produced *in situ*, as will be shown by what follows.

(3) Abundant evidence has been adduced in this work to show that the first stage in the life of Ciliated Infusoria is often not very different in appearance from that which they subsequently assume when (sated with food or in unfavourable media) they become quiescent and undergo encystment. The mode in which Ciliates appear in the pellicle on organic infusions, or as results of the segmentation of encysted Amœbæ (Pl. ix., figs. 93-94), makes this clear. But the former must not be mistaken for the latter condition. Close aggregation within an envelope is quite consistent with their primary phase of existence, when they

appear as products of the segmentation of some larger matrix; but it is totally inconsistent, and contrary to all experience, for Ciliates in the latter phase, or stage of encystment, to be found thus closely aggregated within an envelope.

(*b*) On the Transformation of Hydatina Eggs into Monads, into Mould, or into Bacteria.

In some cases, the substance of Hydatina eggs that have been placed in the experimental pots have become converted into hundreds of *Monads*, in others into a mass of *Mould*, and in others still into legions of *Bacteria*.

The first of these changes has been referred to on p. 52. As I have there said, where it occurs the "peripheral portions of the egg become comparatively translucent, and may be seen to be converted into what appears to be a congeries of very minute hyaline vesicles, each containing one or more granules." In other eggs this peripheral portion may be seen to have resolved itself into a swarm of minute and active Monads, while a similar change spreads through the remainder of the egg substance. In some of such eggs a mixture of Micrococci and Monads is produced, rather than Monads alone. This latter combination has been found, on several occasions, instead of the transformation of the egg substance into Ciliates.

In other cases, Hydatina eggs taken from the experimental pots on the second, third, or fourth day have been found to be full of *Mould*, in the form of a delicate mycelium, such as is shown in Fig. 191, A, (× 250). This is a most common condition—one, that is, in which the entire egg substance is replaced by the mycelium, though none is to be seen on its outer surface or in its immediate neighbourhood. In other cases, hyphæ may be observed which have grown through the envelope in various situations, as in B (× 250). Hydatina eggs in this condition have, as I have said, been very frequently taken from the pots, and what I have seen has convinced me that the early stage of this change is almost indistinguishable from a very early stage of the transformation of the egg substance into an Otostoma. There is the appearance as of very minute vesicles forming throughout the substance of the egg, but the great thickness of such a body makes it impossible to follow the details of the transformation.

A more certain way of obtaining this transformation of the Hydatina egg into a mass of Mould—that is, with freshly laid eggs, not eggs which have already begun to develop into Rotifers —is the following: Place some fresh eggs, together with three minute fragments of a cover-glass in order to protect them from pressure, in a drop of tap water beneath a $\frac{7}{8}$ inch cover-glass, and

then keep this microscope slip in a damp and dark chamber.[1] At the expiration of three or four days, if the eggs have been really freshly laid, I have almost invariably found one or more filled with this mycelium, always of the same kind, though no Mould or spores may be seen outside.

When I have the opportunity again, I shall take eggs from the same stock, treating some as above mentioned and leaving others freely exposed to air and light by placing them in a drop of the same water on a hollowed glass slip, and putting the slip in this condition (that is, without a cover-glass) in an ordinary glass Petri dish containing a thin stratum of distilled water. Examination of each set of eggs would probably throw light upon the influence of physical conditions in the production of this transformation. I venture to predict that none of the eggs in the Petri dish would be found to contain Mould.

The resolution of the substance of the Hydatina egg into a seething mass of *Bacteria* has also been seen, not unfrequently, in eggs taken, after the expiration of three or four days, from the closed pots. Such a specimen is shown in Fig. 191, C ($\times$ 250), in which, throughout the greater portion of the egg, the Bacteria were motionless and imbedded in its substance; though, anterior to the application of a solution of osmic acid, the Bacteria were seen in swarming movement in one region where the egg substance had undergone liquefaction.

Bacteria undoubtedly exist in abundance outside the egg membrane in these cases, and it is therefore easy to assume that such changes are results of infection. It is impossible to prove that they are not so; but what tells against this interpretation is the fact that motionless Bacteria make their appearance in myriads uniformly distributed through the semi-solid egg substance, just as they do occasionally in specimens of Pseudospora like that shown in Fig. 171, C. This is not the kind of thing that would be likely to be seen if infection from without had occurred in one or more sites. Instead of a uniform appearance and diffusion of motionless organisms throughout the whole mass, we should, in the case of infection, expect to see local aggregations of active organisms, and these gradually spreading throughout the substance of the egg. This, however, is an appearance that is never met with in the Hydatina eggs. It looks, in fact, as if this change is one of a series; and that, under the influence of this or that set of external conditions, the substance of a Hydatina egg may become metamorphosed into one or more Ciliates of varying forms; into Monads, alone or mixed with Micrococci; into a heap of Mould; or into Bacteria.

[1] I use Artists' saucers for this purpose, in the manner described on p. 135.

But much stricter proof as to the heterogenetic origin of Moulds and of various kinds of Bacteria will be found in the following section.

XXIII.—ON THE ORIGIN OF BACTERIA AND THEIR ALLIES BY HETEROGENESIS.

If we turn now to the question of the origin of Bacteria and their allies by Heterogenesis we shall find, I think, that the evidence is overwhelming in regard to its reality, though it lacks that kind of certitude which obtains in regard to the heterogenetic origin of some much larger organisms whose birth from strange ancestors we have been following in some of the preceding sections. We may, for instance, as I have shown, see the whole substance of a large Rotifer's egg segment into a number of smaller parts, and we may see such segments presently become active as Amœbæ, Monads, Peranemata, or even as Ciliated Infusoria.[1]

But this kind of proof is impossible in the case of Bacteria. Where the proof is most direct (even where the birth of Bacteria seems to take place under our eyes) it must always be a question of particles of living matter emerging from the region of the invisible—appearing, that is, where previously visible particles were absent. While in other cases, without being so directly present as it were at their birth, we may find them existing in parts of animals or plants, after these have been subjected to certain experimental conditions, where previously, in accordance with all existing knowledge and belief, neither they nor their germs are to be found. They may appear, that is, when such animals or plants, or rather portions of them, have been exposed to certain experimental conditions unfavourable to their pre-existing vital processes, but which yet, as we assume, allow the constituent protoplasm to die more or less slowly, and portions of it to individualise themselves and grow into this or that form of microorganism.

The assumption here is that we have, as starting points, to do with pre-existing units of living matter. Still in the cases where our observations are made upon protoplasm which appears to be actually structureless, or upon one or other of the fluids pertaining to an animal or plant, the actual mode of origin, so far as appearances go, may be precisely similar to that which would occur in Archebiosis: in each case there would be the appearance of particles where none previously existed. The difference between the two cases would be this. In Archebiosis we should have to do with the actual birth of units of

[1] See pp. 31 and 46.

living matter—with a synthesis, that is, from its elements. But in the case of Heterogenesis we have confessedly to do with living matter already existing, so that we postulate only an individualisation of living particles or of larger units, together with a change in their mode of life.

Where the particles of living matter so individualising themselves are so small as to be beyond the range of our most powerful microscopes, it would be impossible to say, as they grow and become recognisable in organic fluids, whether newly appearing particles had arisen by Heterogenesis or by Archebiosis. On the other hand it must be recognised that there are particles of various kinds in the tissue elements and fluids of an animal or plant which it is often impossible by mere microscopal examination to discriminate from germs of microorganisms. And until such germs begin to grow and assume specific shapes, or particular collocations, their discrimination from particles which are normal constituents of such tissues or fluids cannot safely be made.

Whenever, therefore, Bacteria or their allies appear in the midst of the tissues or fluids of animals or plants two possibilities have to be considered in order to account for their presence. The two questions that have to be asked are these :—

(*a*) Are the bodies of plants and animals interpenetrated in all parts by invisible germs of microorganisms, or are they germless?

(*b*) Have the microorganisms which may be found in the tissues or fluids of plants and animals under various conditions been produced therein by Heterogenesis (possibly in the fluids by Archebiosis), or is their presence invariably to be ascribed to infection from without?

(*a*) During the last thirty years it has been commonly held in accordance with the teachings of Pasteur, Lister and others that the tissues and fluids of healthy animals and plants are germless, and altogether free from microorganisms.

In regard to animals, however, it is clear that this position is one which cannot be accepted without very important limitations. It is obvious that microorganisms may, like other particles, get taken up from the mucous membranes of the alimentary canal and the respiratory system, and pass by means of lymphatics into the nearest glands. If they should get through these and ultimately pass into the blood, the generally accepted view is that they are speedily destroyed in this fluid. This view is based upon the fact that bacteriologists are never able to get evidence of the existence of microorganisms or their germs by the inoculation of different suitable and sterilised media, with

blood drawn from a healthy man or from one of the lower animals similarly free from disease.

Yet although the blood and internal tissues of healthy animals and of man are declared to be free from microorganisms and their germs, such organisms will habitually show themselves after death, in the course of a few days, throughout all the organs —even when life has been abruptly terminated in a state of health. It cannot reasonably be said in explanation of this that the organisms naturally present in the intestinal canal have been enabled to spread through the body so as to reach the most remote parts *after death*—since many of the organisms are motionless, and others exhibit mere to-and-fro movements of a non-progressive character. The blood, again, has ceased to circulate, so that this fluid, germless during life, cannot after death be considered to act as a carrier. But if the organisms themselves cannot make their way through the tissues and travel far within brief periods, and if no carrier exists, they must naturally have been born in or near many of the sites in which after death they are speedily to be found.

If bacteriologists are right in their view that the blood is germless in healthy animals because any germs or organisms obtaining access thereto are straightway killed, there seems no escape from the foregoing conclusion.

It has, however, been held by Tiegel, Burdon Sanderson and others that though the blood is germless, "those parts of the animal body which are in closest proxmity to absorbing mucous membranes are most liable to be found pregnant with microphytic life when tested by suitable methods."[1] Their experiments showed indeed that such organs as kidney, spleen and liver, when removed from the body of a healthy animal immediately after its death, and suitably treated, could always be made to reveal the presence of microorganisms.

But cutting out portions of internal organs of recently killed animals, enveloping them with superheated paraffin, and then placing them in an incubator at a suitable temperature, followed by the finding of swarms of Bacteria in the central red and uncooked portions is not a method that can possibly give us certain information as to the mode of origin of the organisms found. It may be, as Burdon Sanderson and others concluded, that the organisms found came from "germs which existed and retained their latent vitality in the living tissues"; but it is equally possible, as I maintain, that they may have had a heterogenetic origin within these tissues themselves.

It seems perfectly clear that experiments of this kind, if carried no further, could teach us nothing decisively; that their

[1] *British Medical Journal*, 1875, vol. i., p. 199; see also 1878, vol. i., p. 119.

results, in fact, are of no more value than those that may be obtained by the examination of the brain and its membranes three or four days after a healthy animal has been killed. There also swarms of microorganisms would be found, as I can testify; and if bacteriologists are right that organisms and their germs are, as they say, "destroyed" in the blood, we could only conclude that the organisms so found must have been produced by archebiosis or by heterogenesis.

In regard to plants, that is fruits and vegetables of different kinds, the case is not so complicated and Pasteur may have been perfectly right in declaring that, when healthy, their tissues are germless. Thus, in considering the interpretation of cases in which microorganisms are found in the interior of certain vegetables or fruits after they have been submitted to various unnatural conditions, the question will not be whether we have had to do with the wakening up of latent pre-existing germs, but rather whether the organisms found are results of an infection that has recently been brought about — that is, during the exposure of the vegetables or fruits to the experimental conditions. And this brings us to the consideration of the second of the two possibilities above referred to.

(*b*) The second possible mode of accounting for the presence of microorganisms in the tissues of healthy animals and healthy plants is that they have resulted from some process of infection brought about antecedently to, or during the continuance of, the experimental conditions to which they have been subjected.[1]

It is of great importance for the proper consideration of this possibility that we should have some definite knowledge as to the power possessed by such microorganisms as Bacteria and their allies of penetrating the healthy tissues of plants and animals, as to the means by which they are enabled to do so, as well as concerning the time needed for such an operation. Fortunately one such investigation, very much to the point, can be referred to.

[1] I say "antecedently" because the explanation favoured by Burdon Sanderson of the finding of swarms of Bacteria in kidney, spleen and liver in his experiments previously referred to is some process of infection from the alimentary canal. How the infection is brought about he did not think it necessary to explain (see *Brit. Med. Journ.*, 1878, i., p. 120), that is, how the Bacteria (not to speak of their germs) could penetrate all the coats of the intestine and the capsules of the abdominal organs; nor why, if Bacteria or their germs could be active enough and have the power of effecting such a migration, they should, on arrival in this or that organ lose all their activity and pass into a "latent condition."

M. C. Potter[1] has carefully investigated a Bacterial disease of the turnip which he calls "White Rot," due to infection by an organism named *Pseudomonas destructans*. He shows that this Bacterium "secretes an enzyme which dissolves the middle lamella and causes the softening and swelling of the cell-wall," and also a toxin. Later, he enters into details as to the mode in which the Bacterium attacks and penetrates the cells of the turnip—the latter process being actually seen.[2] These latter observations were made upon a small fragment of turnip inoculated with a pure culture of *P. destructans* and introduced into a hanging drop. The influence of the Bacteria was very rapidly brought about. One particular cell attacked by the Bacteria was watched; a wall common to it and the next was measured, and after half an hour it was found to be nearly twice as thick as before; in another twenty minutes it was nearly three times as thick; in an hour and a quarter from the commencement the two parts of the cell-wall began to separate, and in another quarter of an hour there was a distinct gap between them. By this time, also, all the protoplasm had separated from the cell-wall, and formed an irregular bag in the centre of the cell—owing to its death under the action of the toxin. "After this the changes were less rapid, and beyond a slight further separation of the cells, a *more rotten and watery appearance of the cell-wall* was all that could be observed." Contiguous cells were being attacked in the same manner.

"The Bacteria continued swarming around the cell-walls," the author says, "and next morning (by which time the cells had been destroyed several layers deep) many Bacteria had come to rest in contact with the wall, their long axis being perpendicular to its surface; and one or two had the appearance of being imbedded in the wall as if in the act of boring their way through." The actual penetration of the wall was subsequently watched on several occasions. "The time required varied with the thickness of the wall, but on an average occupied about three hours." But Potter found that this Bacterium "had no power to penetrate the cuticle of the mature epidermis of the turnip"; it was also incapable of penetrating the epidermis of mature leaves, the fully developed cuticle being proof against the action of the enzymes excreted by *P. destructans*, though young leaves from the growing points "possessing little or no cuticle" were found to be vulnerable.

The important facts made known by this research are, therefore, these: the vegetable cell is only capable of being penetrated when its walls are not thick and hard originally, and when they have been extremely softened by long contact with the cytase excreted by a number of Bacteria—the need of a conjoint attack is distinctly indicated by the author who says[3]: "Very soon the number of individual Bacteria becomes largely increased, each one contributes its share of toxin and cytase, and in a very short time these products have sufficiently accumulated to kill the first cell. . . . It is not, however, until the protoplasm has been killed and the cell-wall very much softened that the Bacteria have the power of perforating the walls and passing into the cell cavity. It would hardly be supposed that a single Bacterium, through its own excretions, could soften the wall and pierce it at one definite point after the manner of a fungus germ-tube. The extreme minuteness of the Bacteria and the rapidity of their multiplication lead them to act, as it were, in concert, and the wall becomes softened by the cumulative action of many Bacteria before the penetration of a single individual."

The mode in which a Mould infects and penetrates a vegetable cell is very similar, allowance being made for its greater size, which permits a single individual to do what can only be brought about by numbers of organisms in

[1] *Proceed. of Roy. Soc.*, vol. lxvii., p. 442.
[2] *Loc. cit.*, vol. lxx., p. 392.
[3] *Loc. cit.*, p. 395.

the case of Bacteria. This subject has been recently investigated by Nordhausen[1] while studying the parasitism of *Botrytis cinerea.* I quote from Potter, who says:—

"He has shown that the spore of this fungus excretes a powerful toxin in its initial stages of germination before any trace of the germ-tube can be detected. Its manner of effecting an entrance into a host plant is first to kill the cell by the emission of the toxin; the germ-tube then penetrates the dead cell and is nourished saprophytically upon it; with the vigour thus gained it destroys the neighbouring cells and passes from one to another without further difficulty. The fungus hypha has the power of penetrating the cuticle, *but only in young and tender structures;* old and hardened membranes could only be entered when the cuticle had been injured, or when it had gained strength by special saprophytic nutrition."

These results will prove important as standards for comparison with others to which I am now about to refer. We have seen that in the case of actual infection by Bacteria there is need for the co-operation of many organisms in order to bring about, by their secretions, the softening of the wall of every single cell that is penetrated; that some time is required for the operation; and that the softening produced must be considerable before any such penetration is possible. It will be seen how very different is the state of things in cases which I shall cite as instances of the origin of Bacteria and their allies, in the tissues of plants and animals, by a process of Heterogenesis.

The presence of two characteristics, wherever they co-exist, may be regarded as strongly in favour of the interpretation of Heterogenesis as against Infection, as the following remarks will show.

(1) The means adopted by Bacteria for bringing about the penetration of cells are such as are associated with the vital processes of adult organisms. So that there is no reason to think that invisible or scarcely visible germs of such minute organisms would have the power of secreting a cytase sufficient in amount to bring about that degree of softening of a cell-wall which has been found to be a necessary preliminary to their penetration. *Yet in multitudes of cases it is minute germs of Bacteria and their allies which may be seen developing within cells or tissues.*

(2) Again, the process of infection, as described by Potter, is one brought about by active organisms which affix themselves to a cell-wall until it becomes softened, and then succeed, by reason of this same activity, in boring their way into the cavity of the cell. On the other hand in very many of the cases in which, as I maintain, Bacteria and their allies may be presumed to be originating by Heterogenesis, what can often be seen is this—particles becoming visible in the midst of homogeneous protoplasm; such particles being invariably motionless but

[1] *Jahrbücher für Wissensch. Botanik,* vol. xxxiii., 1899.

followed soon by the appearance of definite Bacteria or their allies, recognisable as such by their shapes and modes of collocation. *But these Bacteria or their allies, like their germs, are invariably, at first, found to be motionless.* This primary motionless condition is, in fact, the rule in the case of organisms taking their origin by Heterogenesis. Thus, when Monads are formed in the pellicle on a hay infusion they are at first motionless; when Amœbæ or Actinophrys are developed from the substance of large Confervoid cells or from resting spores of Spirogyra; and again when Amœbæ, Monads, Peranemata, or Ciliated Infusoria arise from the transformations of the substance of the egg of this or that Rotifer—in all these cases also the resulting organisms are at first motionless.[1]

These two points are, therefore, of great importance. For the purpose of interpretation it should be borne in mind that in cases of Infection by Bacteria and their allies we have to do with *adult organisms in a state of activity;* while in cases where Heterogenesis may be presumed to be occurring we have invariably, in the first place, to do with *germs and motionless organisms.*[2]

I shall now proceed to mention some instances in which it seems clear that Bacteria and their allies have arisen by Heterogenesis. I make a selection, for this purpose, of a few typical cases out of many others which I might have cited.

Bacteria may constantly be seen developing in the way I have mentioned within the living cells and filaments of various *Algæ.* In Vaucheria and in Spirogyra this is commonly to be seen where the plants have been kept in unnatural conditions for a time; shut up, for instance, either in a cupboard or within a stone pot. In the case of Vaucheria they may often best be recognised in and near the growing points of the filaments where they show themselves first as mere motionless specks, which gradually develop into Bacilli, and after a time take on an active existence. In the filaments presenting these appearances the wall may appear quite healthy, showing no signs of softening, nor is there any indication whatever of the penetration of Bacteria from without. The development of Bacteria within the cells of Spirogyra may be best seen in cases where the endochrome is not very abundant and the cells are small. Motionless, colourless particles seem to bud from the edges and surface of the bands

[1] See pp. 5, 8, 10, 31, 46, 69.

[2] Of course by "germs" I mean here merely minute and undifferentiated stages of the organisms in question, produced by Heterogenesis, and not the ordinary acceptation of the word, viz., a reproductive unit formed in an organism of like kind.

of endochrome, and some of these gradually take on the forms of Bacteria and begin to exhibit swarming movements. This may be commonly seen in cases where the cell-wall presents a perfectly healthy appearance, and where there is absolutely no indication of infection taking place from without.

The same kind of thing is often to be observed within the thick-walled resting spores both of Vaucheria and of Spirogyra. There is the appearance of motionless particles in some part of the spore, the appearance of Bacteria in the midst of these particles, and the gradual assumption by the Bacteria of swarming movements. Observations of this kind were not unfrequently made in cases where these resting-spores had been undergoing one or other of the changes previously described, and very similar observations have been made upon Hydatina eggs and encysted Amœbæ, as mentioned at the close of the last section.

Again, I have occasionally seen a development of motionless Micrococci and Bacilli taking place inside the thick wall of a Nitella cell, between it and the chlorophyll layer, such as I have shown in Pl. xviii., fig. 192 (× 700). Yet the normal cyclosis was still going on within this cell, showing that there could be no apertures or solutions of continuity of any kind; and all the microorganisms to be seen in different stages of development in this layer were quite motionless, so far as could be observed. Their imperfect definition in the photograph, however, makes it possible that absence of spontaneous movement was not absolute, unless the slight blurring may have been due to vibrations during the prolonged exposure needful to enable the light to pass through the whole thickness of the filament. Their appearance along a particular band of the filament only is, of course, due to the parts on either side being out of focus.

I have also endeavoured to throw light upon this question in another way, that is, by repeating, with variations, some of the experiments of Lechartier and Bellamy by which they studied the fermentation that occurs in various vegetables and fruits when shut up within hermetically sealed vessels. They showed that the oxygen of the air was soon consumed by the vegetables or fruits, which then began to break up sugar, to give off carbonic acid, and to produce alcohol and acetic acid. They came to the conclusion in 1872 that this fermentation might certainly occur without the production of the alcoholic ferment.[1] They in fact adopted Pasteur's view that the formation of alcohol in these cases was due to the altered activity of the cells of the fruit, which, in the absence of free oxygen, act after the fashion of ferments. In a later communication,[2] however, these investi-

[1] *Compt. Rend.*, 1872, ii., p. 1203.

[2] *Loc. cit.*, 1874, ii., p. 1006.

gators stated that in their experiments with potatoes and beet-root, while alcohol and carbonic acid were produced in the same way as with the fruits, and the alcoholic ferment was absent as before, Bacteria of different sizes were invariably found in the acid fluid which impregnated the softened tissues of the vegetables in question. No details on this point were given, and the authors do not appear to have made any further observations on the subject; nor did Pasteur offer any reply to such statements, though he had, about this time, been working at the subject himself.[1]

Having determined to endeavour to obtain some more definite information as to the appearance of Bacteria in this way I have, during the last two or three years, made various experiments in which, after small *Potatoes* had been carefully washed they were allowed to soak for a time in different germicidal fluids. First of all I employed a solution of mercury bichloride (1 : 2000); while later, after the preliminary washing, the potato was allowed to soak in a five per cent. formalin solution. The screw-top bottle in which the potato was placed was also thoroughly washed out with one or other of these fluids. In these cases organisms were found within, but also after a time on the surface of, the potatoes thus treated; so that these particular experiments and methods were rejected as not yielding trustworthy results. This was necessary because at a rather earlier date Pasteur had stated that in experiments which he had made with fruits no ferment organisms ever appeared. He declared again that the tissues of healthy fruits and vegetables were germless, but intimated that unless care was taken they might make their way in from without.[2]

Subsequently I used a stronger solution of formalin, and have never since found organisms on or near the surface, though they have often been found within cells in the central portions of the potato. I will now, therefore, give brief details of some of these experiments.

In July, 1901, after well washing a small new potato it was allowed to soak in a ten per cent. solution of formalin for ten minutes, in a small screw-top bottle, and during this time the fluid was frequently shaken so as to cover the whole inner surface of the bottle. At the expiration of the time named the top was unscrewed, the fluid poured out, and the top then tightly refixed, leaving the potato itself and the inner surface of the bottle wet with the formalin solution. The bottle was subsequently left in a cupboard for seventeen weeks, the temperature of which for

[1] *Compt. Rend.*, 1872, ii., p. 788.
[2] *Loc. cit.*, 1872, ii., pp. 788 and 981-2.

a long time remained about 70° F., though it afterwards fell to 50° F.

When removed from the bottle at the expiration of this time the potato was found to be quite firm and not at all shrunk. On section it was seen to be discoloured to a pale earthy tint, with mottlings here and there of a rather darker colour. The cut surface was moist and had a distinctly acid reaction, and there was not the least sign of softening or disintegration anywhere. Thin sections having been made, they were shaken up in a small tube with distilled water, so as to get rid of the starch-grains from many of the cells, and the sections were subsequently allowed to soak in some of Westphal's mastzellen stain, diluted with two per cent. formalin for two hours.

On microscopical examination of these sections, groups of Bacteria were found in large numbers of the cells, though not in those near the surface. The contents of one of these cells is shown in Pl. xviii. fig. 193, B (× 500); some of the Bacteria were free and others were in, or lying on, the primordial utricle, but, as I have usually found with microorganisms in such situations, they were not appreciably stained. Some cells, which did not contain obvious Bacteria, showed plenty of minute cocci-like bodies on the surface of the primordial utricle, also not taking the stain, which probably represent early stages of the Bacteria (Fig. 193, A, × 700).

Another larger potato, about two inches in diameter was treated in exactly the same way as the last in September, 1901, and after the bottle was finally closed it was left on the surface of an incubator at a temperature of about 80° F. for seven weeks.

When examined the potato was not found to have shrunk, or to be appreciably altered on the surface. On section, it was moist, of acid reaction, and showed as before a pale earthy colour with rather darker mottlings.

Sections were made and treated in the manner previously indicated, and on examination multitudes of Bacilli were seen here and there in cells in all parts of the section except for about one-fifth of an inch from the surface. In places also there were fine mycelial filaments containing spore-like bodies. Some of these Bacilli took the stain fairly as may be seen in Fig. 194, A (× 500), in which the two kinds of organisms are shown. In or on the primordial utricle also there were multitudes of very delicate interlacing filements (? Bacilli), containing an abundance of spores which had taken the stain freely, as may be seen by Fig. 194, B (× 500).

In November, 1900, a small new potato had been treated in the same manner that I have already described, but after pouring away the ten per cent. formalin solution, the bottle was filled with

carbonic acid gas before screwing on the top.[1] This bottle was then placed within an incubator and allowed to remain there at a temperature of 84° F. for six weeks.

On examination the appearance of the potato externally and internally was almost exactly such as I have described in the others. There was the same mottled colour of the cut surface, with a rather deeper tint in the centre as well as in some other parts.

Sections of the central portions of the potato were made and placed for a short time in a carbo-fuchsine solution. On microscopical examination in many of the cells very small mycelial filaments (something like Crenothrix filaments), with spore-like bodies at intervals, were found, such as are shown in Fig. 195, B (× 500). The filaments were lying on the primordial utricle, and as in many other cases the organisms had scarcely taken the stain at all. In other cells what seemed like the beginnings of such organisms, or of others very similar, were found on the surface of the primordial utricle, as shown in Fig. 195, A (× 500).

At the end of March, 1903, I made another slight variation in the conditions, and again obtained a rather different result. A small new potato, after careful washing, was placed in a small tin with a very tightly fitting cover and allowed to soak in ten per cent. formalin for twenty minutes, the fluid having been shaken about several times so as thoroughly to wet the whole internal surface of the tin. The fluid was then poured off, leaving the surfaces wet with the solution as before; the tin cover was very tightly jammed down and the vessel was again placed within a copper incubator at a temperature of 75° F., and allowed to remain their for eight weeks.

When taken out and examined the cut surface of this potato presented just the same characters as in the others; the whole substance was firm throughout, there was no shrinking, and the central portion was rather darker than the other parts which showed the usual mottling.

Sections were made, shaken up in distilled water in a small tube as before, and then placed for a short time in a dilute gentian-violet solution. On microscopical examination a large number of the cells scattered throughout the sections were found to show the most delicate branching tufts of a new kind of microphyte, probably a species of Cladothrix, which had taken the stain slightly, and such as are shown in Fig. 196 (×500). These tufts were mostly seen to be sprouting from the external surface of the primordial utricle, where it had shrunk away from the cell wall.

[1] The filling the vessel with CO_2 was a method adopted by Pasteur in his experiments on the fermentation occurring in fruits contained in closed vessels.

No Bacteria, and no ordinary Mycelia were found in either of the sections, though they were most carefully examined.

A few experiments have also been made with small *Turnips* about two inches in diameter, to two of which I will now refer.

A perfectly sound turnip of the size mentioned was, in November, 1901, first well washed in water and then allowed to soak in a screw-top bottle in a ten per cent. formalin solution for ten minutes. It was subsequently treated in exactly the same manner as the potatoes had been. After the top of the bottle had been tightly screwed on it was left on the top of the incubator at a temperature of about 80° F. for seven weeks.

On examination at the expiration of this time, the turnip was found to be somewhat shrivelled in its upper two-thirds. The odour of the bottle was disagreeable and pungent, though slightly aromatic and spirituous. The odour was so strong that it did not seem likely that the shrivelling was due to evaporation, owing to the screw-top not having been quite air-tight.

On section the rather shrivelled upper two-thirds was found to be much discoloured and honeycombed; the lower third being much less so. Sections were made and soaked in dilute mastzellen stain; and on examination cells here and there, not continuously, but in the upper and lower portions alike, were found to be crowded with very minute Bacteria, most of which took the stain only slightly. In Fig. 197, A (× 500), a large aggregate of these organisms is to be seen, with others scattered about over contiguous portions of the section.

Another small turnip of the same size as the last was, at the same date, after being well washed, placed in a screw-top bottle and stood on a small earthenware pot, in order to protect it from six drachms of pure formalin which had previously been poured into the bottle. The top was then tightly screwed on, and the bottle was placed on the incubator by the side of the other at about 80° F. where it remained for eight weeks, the turnip being in an atmosphere saturated with formalin vapour.

On examination this turnip was likewise found to be slightly shrivelled, and it was rather soft and doughy to the touch. On section the colour was almost natural except for a depth of about one-third of an inch round the periphery, where it was slightly discoloured, and in the centre where there was a small area about one quarter of an inch in diameter which had a rather gelatinous appearance.

Two sections through this central region and its neighbourhood were made, and then soaked in dilute mastzellen stain. On microscopical examination they were found to contain moderately large Bacteria, mostly in small groups, in a large number of the cells; though here and there larger masses of

Bacteria were found, such as are shown in Fig. 197, B (×500). In many of the cells the Bacteria seemed to be developing in and on the surface of the primordial utricle, and also within and on the surface of the nuclei of the cells.

I have made only one experiment of this kind with an *Apple*; and in this case a rather small but thoroughly sound specimen was placed in a screw-top bottle, standing on a small earthenware pot as before so as to remove it from contact with some pure formalin which was placed at the bottom of the bottle. The top was tightly screwed on, and the bottle was then placed in a cupboard where it remained for eight weeks, the temperature of the cupboard varying during this time between 70° and 56° F. The apple was thus left, as the turnip had been, in an atmosphere saturated with formalin vapour.[1]

On examination the surface of the apple was found to be hardened, and on section irregular patches of brownish discolouration were seen. Otherwise nothing abnormal was observed.

Microscopical examination of an unstained section showed, in a few of the cells, a small Fungus mycelium, such as may be seen in Pl. xviii., fig. 198, A (× 250). Specimens like this were found in cells in different parts of the section, though in the majority of them nothing of the kind was met with. When present the growth seemed to start from the cell-wall close to the nucleus, if not from the nucleus itself. No Bacilli were seen; but in some cells what appeared, judging from their uniform size and mode of arrangement, to be a number of Micrococci were found on the primordial utricle, such as are shown in Fig. 198, B (× 375). They scarcely stained at all with carbo-fuchsine.[2]

I have now to record an interesting case of spontaneous change in some apples, which occurred under the following circumstances. Last autumn I received from a friend in America a case of very choice Canadian apples. The case had a separate cardboard partition for every apple, and they were all in excellent condition. Some of them were kept as late as the second week in January of this year. From about the middle of December I noticed that many of these apples when cut through the centre showed a brown discolouration beginning at a number of separate points around the periphery of the core, as may be seen in

[1] I have not had many successes with this method, and do not recommend it—especially as the soaking for a time in ten per cent. formalin has proved to be perfectly sufficient to guard against external contamination. It is difficult to tell how far the formalin vapour penetrates into the substance of fruits or vegetables left in an atmosphere saturated with it for many weeks.

[2] As the magnification is low the use of a pocket lens will make these organisms more distinct—especially those near the centre of the figure.

Pl. xix., fig. 199, A and B (½ nat. size). An early stage of the change is shown in A and a more advanced stage in B. In all other respects the apples were perfectly sound and of a delicious flavour, and none of those eaten anterior to the date mentioned showed any unusual appearance. Towards the end of December the above photographs were taken; and on consulting Lindley's "Vegetable Kingdom," it became plain from a figure there shown (p. 559), that these points of change occurred at the junction of the ovarian and the calycine portions of the fruit.

I examined portions of the altered tissue under the microscope, fully expecting to find some Mould as the cause of the change. But, much to my surprise, after a tolerably careful examination, I was unable to feel sure that organisms of any kind were to be found in the tissue which had become thus altered. Subsequently I tried to stain some sections, and made a still more careful examination, with the result that I found on or in the primordial utricle of many cells cocci-like bodies looking like the germs of microorganisms. But as their nature seemed doubtful I took two of the apples to Dr. Allan Macfadyen and asked him kindly to see whether any microorganisms could be developed from this altered tissue of the apple. On January 5th he wrote to me, as follows: "I was unable to detect the presence of Bacteria in the Canadian apples you left here, by microscopical examination. I accordingly made a number of subcultures, but in no instance have I succeeded in obtaining a growth."[1]

I had by this time only two or three of the apples left, so I placed them in the incubator at a temperature of 76° F. and there left them for eight days. On section two of them were found to present the brown discolouration in the usual situations to a well-marked extent. Some portions of the brown tissue were broken up with needles, placed in a dilute solution of the mastzellen stain, and were afterwards submitted to careful examination with the microscope. There was certainly a very distinct increase of the cocci-like bodies in the primordial utricle, remaining unstained, as in Fig. 199, C (× 700), though all the other granules in the cells had become strongly stained. In some places the cocci were seen in distinct rows, branching and crossing one another as in Fig. 199, J (× 375), in the neighbourhood of the letter, so that they looked like spores within minute filaments.[2]

[1] Some time previously Dr. Nabarro, of University College, had been similarly unsuccessful in obtaining growths from a potato which had been treated in the manner I have detailed on p. 299, and in which organisms seemed to be present in an early stage. Such lack of success after trials with a few culture media is, of course, far from disproving the presence of microorganisms.

[2] Unfortunately this particular photograph was taken at a low magnification, but C and each of the others was taken at 700 diameters.

A further careful and prolonged examination revealed the fact that very many of the cells showed, in whole or in part, on or in the lining membrane, the cocci-like bodies as in C, though in other cells there were none of them. There was often a tendency for these bodies to arrange themselves in rows (as in E and I); and in places to grow into delicate filaments (as in D and F). Such filaments were also seen occasionally crossing the cavity of the cell, and having spore-like bodies at intervals. A few larger filaments or hyphæ, such as G, were likewise seen together with Toruloid corpuscles, as in H. The spore-like body in G, from which the hypha has developed, is only a little larger than one of the cocci-like bodies to be seen near the lower left corner of C. Any doubts as to the reality of these latter bodies being embryo Bacteria may be set at rest by comparing them with what is shown in Figs. 193 and 195. All the organisms found here, as with those shown in the figures above mentioned from other vegetable cells, were similar in refusing to stain with all ordinary dyes.

Although the first examinations of these apples showed, therefore, only very doubtful organisms or none at all, a prolonged search has made their presence abundantly clear, and has shown that the spontaneous changes occurring in so many distinct foci in the very midst of their tissue has been correlated with the origin and development of microorganisms.

I have also made a very few observations on *Tangerine Oranges*, to two of which I will refer. In February, 1901, two of these oranges were placed in a screw-top bottle and soaked in a ten per cent. solution of formalin for fifteen minutes. The fluid being also shaken about several times so as to wet the whole inner surface of the bottle. After the fluid was poured off and the cover tightly screwed on, the bottle was placed in a cupboard for fifteen days, the average temperature of which was about 50° F.

When the first of these oranges was cut through in a longitudinal direction, a slight mouldy odour was at once perceived, and in the central white tissue and around the pips there was a greenish black mass of mould. This was strictly confined to the central parts of the orange, and nowhere came within three-fourths of an inch of the surface. The skin generally was perfectly sound, though it had become hard and was of a slightly brownish colour from the action of the formalin. Microscopical examination of the more peripheral parts of the orange also showed no mycelial filaments or organisms of any kind.

The other orange in the same bottle showed no organisms either to the naked eye or on microscopical examination.

Soon afterwards two other Tangerine oranges were treated in

the same way, and subjected to similar conditions, except that they were left in the bottle for a much longer period—that is, for five and a half weeks instead of only fifteen days. About five days before the bottle was opened one of the oranges was seen to show a patch of dark colour on one side, and when it was subsequently cut open longitudinally all the central white tissue was found to present an altered appearance being of a rather dirty white colour; and on microscopical examination it was found to be densely infiltrated with a delicate Fungus mycelium. The seeds were discoloured, and the mycelium was also found to extend into the yellow substance of the orange. In one place there was a patch of a blackish colour, and this was found to have grown into the rind of the orange at the point where the discolouration on the surface was seen. It had not, however, actually reached the external surface, it had evidently grown from within outwards, and the surface of the orange here and elsewhere showed no trace of Mould of any kind.

The companion orange again showed no organisms either internally or externally.

There is no means of accounting for Mould springing up in the interior of an orange by infection from without. In a memoir entitled "Recherches sur la pourriture des fruits"[1] Davaine points out that in fruits, such as the apple, the pear and the medlar in which there is an open calyx, "le tube calicinal peut conduir les spores ou leurs filaments jusqu' au centre du fruit. C'est ainsi que se produit le blettisement,[2] qui n'est autre chose qu'une pourriture"; but the process of rotting, he says, *is always external* "chez les fruits qui sont partout recouverts d'un épiderme, tels que le citron, l'orange, et les fruits à noyau."

In the case of the apples to which I have referred there was clearly no such process of infection from within as that to which Davaine refers. In the Canadian apples the change occurred simultaneously in many points almost as much removed from the seed cavities as from the surface of the apples; and a comparison of what was found in the primordial utricles of the cells with what has been found in similar situations in the potatoes, as shown in Figs. 193 and 195, leaves little room for doubt that what are represented in Fig. 199, C, D, &c., are really germs of microorganisms. While in the other apple delicate Fungus mycelia (Fig. 198, A) were found springing up within various isolated cells in the midst of the substance of the fruit.

[1] *Compt. Rend.*, 1866, pp. 277 and 344.

[2] That is, the mellowing process that occurs in pears and medlars more especially. Further on in his paper Davaine says he has "recently recognised that this latter (blettisement) may take place where spores are excluded, and in the absence of any mycelium."

Again the presence of the Bacteria and other organisms within the cells of the two small turnips and the different potatoes that have been referred to are equally incapable of being accounted for by any process of infection from without. There is absolutely no relation between what I have found in these cases, and an actual process of infection such as M. C. Potter has described (see p. 295). We have to do, in fact, in the cases that I have cited, with motionless germs of microorganisms arising *de novo* in or on the substance of the primordial utricles of isolated cells, having intact walls, and scattered throughout the substance of the potatoes and the turnips in question—in all parts, that is, except in the superficial portions that have been saturated with the germicidal fluid in which they had been for a time soaked.[1]

As I have previously pointed out (p. 299) the existence of "latent germs" in the substance of healthy fruits and vegetables is not assumed—it was, in fact, expressly denied by Pasteur. Hence the great weight to be attached to the preceding observations as evidence that the various microorganisms found within the cells have actually originated there by heterogenesis.

It remains to be seen what evidence of similar cogency can be obtained in regard to the origin of microorganisms within the tissues of animal organisms.

It would be useless to multiply instances. I will therefore first cite a single case in which the origin of Bacteria may be actually watched within the body of a low animal organism, and then turn to their mode of appearance within some of the tissue elements of different vertebrates.

Evidence of a particularly convincing nature is to be obtained from the examination of a little creature low in the scale of animal life, namely, *Cyclops quadricornis*, one of the Entomostraca so commonly to be found in ponds. It may be seen from Pl. xxiv. of Baird's "Natural History of the British Entomostraca"[2] that the four pairs of abdominal feet and also the tail are furnished with a number of "plumose spines or setae."

Examination of one of these organisms will show that within the chitinous envelope of these slender spines, which taper away to sharp points, there is nothing but structureless protoplasm to be seen (Fig. 200, A, × 700). If we take one of these little creatures, put it in a drop of distilled water, on a glass slip with a fragment of a No. 2 cover-glass on each side of it, and place over all a cover-glass, it will be found that the animal is soon

[1] In these cases the organisms often have to be long and carefully searched for. A perfunctory examination would almost certainly lead to the statement that no organisms were present.

[2] Ray Society, 1850.

killed by the weight of the latter though the fragments of glass prevent rupture of the body. We may then place the microscope slip in a Petri dish containing a thin stratum of water (so as to prevent evaporation from beneath the cover-glass) and fixing upon one of the tail setae, which are larger than those on the abdominal feet, we may examine it from time to time. What may be observed is this.

After an interval of two or three days (the duration depending upon the temperature of the air at the time) we may see, under the highest power of our microscope, scarcely visible motionless specks gradually appear in increasing numbers in the midst of the structureless protoplasm; and still later we may see some of these specks growing into Bacteria, as in Fig. 200, B (× 700), which is a representation of A after four days. At last the whole interior of the spine becomes filled with distinct Bacteria as may be seen in C (× 700), which is from a photograph of the same spine on the sixth day—the temperature during these days varying from 70-75° F. Later still, all the Bacteria, previously motionless, began to show active swarming movements.

In such a case it is clear that we have to do with no process of infection from without, but with a *de novo* origin of Bacteria from the protoplasmic contents of the spines or setae. The fact that they appear in these situations as mere separate, motionless specks, and gradually take on the forms of Bacteria (also motionless at first), is, as I have previously indicated, just what we might expect if they had actually taken origin in the places where they appear. On the other hand, such a mode of appearance is totally opposed to what might be expected if the microorganisms had obtained an entry from without, through the chitinous envelope of the spines.

I pass now to what may be regarded as another absolute proof of the hereterogenetic origin of Bacteria, as convincing as that which I have shown to occur within the closed cells of certain vegetables.

I have already pointed out that in many parts of the bodies of man and of higher animals microorganisms are known to exist in abundance. This is the case, for instance, throughout the whole length of the alimentary tract, and throughout almost the whole extent of the mucous membranes of the respiratory tract. It is clear also that some of the microorganisms may be taken up from these mucous membranes by lymphatics, and if they pass the nearest lymphatic glands some of them would ultimately find their way into the blood. When there, the view generally accepted is that the Bacteria and their allies are at once "destroyed." The blood of healthy animals is delared to be germless, and much importance is attached to the germicidal qualities of this fluid.

On the other hand it has been found, as I have previously stated (p. 293), that in organs contiguous to the alimentary canal—such as the kidney, the pancreas, the spleen and the liver—taken from a healthy animal immediately after death, with every precaution needful to prevent contamination from without, swarms of micro-organisms can be made to appear therein at will.

Speaking of such experiments made by himself Burdon Sanderson wrote as follows:[1] "Under the conditions I have described to you, it seems to me quite impossible to suppose either that germs could penetrate to the organ from the outside, or that any germ encountered by the organ in its transference from the body of the animal to the basin could escape destruction. If, therefore, Bacteria be found, they or their germs must have been there before the organ was plunged into the hot liquid. . . . The results of all the experiments whether with liver or kidneys was the same. The soft red kernel of uncooked tissue at the middle of the organ always contained Bacteria, the vigorous development of which was indicated by their large size, countless numbers, and active movements. To my mind the experiment is conclusive."

In reference to this it is right to say that similar results have been obtained by other investigators using either similar methods, or methods equally trustworthy. Such experiments have been made by Tiegel, Billroth, Nencki and Giacosa, Horsley and Mott, as well as by myself.

As I have previously intimated, the finding of organisms under these conditions is a fact so important in view of the theories of Pasteur and Lister, and the general belief as to the germicidal qualities of the blood, that the results require to be most carefully scrutinised.

To postulate the presence of "latent germs" in these abdominal organs and to assign as a reason the close "proximity to absorbing mucous membranes," and nothing else, surely cannot be regarded as a full and satisfactory explanation. It assumes, without proof of any kind, that microorganisms, even in healthy animals, are constantly making their way out through the walls of the intestine, and wandering promiscuously into this or that organ, only in the end to lapse into a condition of "latent vitality." Could anything in the way of explanation be more gratuitous and unsatisfactory?

With a view to throwing light upon this important question by the production of actual evidence I obtained a sheep's kidney from a recently killed animal, and saw a coating of fat nearly an inch thick stripped from it. The whole organ was then left

[1] *British Medical Journal*, 1878, vol. i., p. 119.

to soak for four hours in a two per cent. solution of chromic acid, when it was removed and placed in a bottle still wet with a ten per cent. solution of formalin. The screw-top having been fixed so as to prevent evaporation, the bottle was transferred to an incubator and left at a temperature of 76° F. for thirty-six hours.[1] When the organ was cut the chromic acid was found to have discoloured it to a depth of about a quarter of an inch, but within that margin the kidney substance was red and only slightly softer than natural. There was no distinct odour of putrefaction. A small portion of the organ was cut out and teazed in a drop of a weak solution of gentian violet, and fragments, after a short interval, were carefully examined under the microscope. A comparatively small number of Bacteria were found free, between the separated and broken-up cells, and the cells were densely filled with granular matter, as may be seen in Fig. 201, A (× 700), but it was impossible to identify with certainty any of the granules as germs of Bacteria. Sections that were made and carefully stained gave no more definite results.

Another sheep's kidney from a freshly killed animal was therefore obtained and treated in the same manner, except that it was left in the incubator at 76° F. for three and a half days. When the organ was cut through it was deeply stained at the circumference as before with the chromic acid; but the red tissue within was much softer, and the odour was most offensive and putrid. Portions of the organ were at once put into a ten per cent. formalin solution with a view to obtaining sections therefrom, but a minute portion was cut off as before and teazed in a drop of gentian violet. On examination with the microscope after a brief interval I found the fragments of the tubules and kidney cells full of micrococci which had taken the stain well, altogether with numbers of figure-of-eight organisms and short chains (Streptococci) such as are shown in Fig. 201, B (× 700).

In a few days Dr. J. S. Collier kindly sent me a number of sections, stained and unstained, which he had been good enough to cut from the portion of the kidney in the formalin solution which I had handed over to him. Some of the specimens sent to me had been stained with methylene blue or with logwood, and then mounted in balsam; while I stained some of the plain sections with gentian violet, and subsequently mounted them in glycerine. On the whole, rather more details could be made out with these latter sections than with the specimens mounted in balsam.

Every section, however, showed, inside the area which had

[1] I was using the incubator for other purposes at this temperature, and therefore did not alter it.

been affected by the chromic acid, that almost every cell within the renal tubules was full of developing or actually developed Bacteria. The former appeared as mere cocci-like particles which had taken the stain like the developed Bacteria, both being situated in the midst of cell granules comparatively unstained. The organisms were distributed through the substance of the cells, but often seemed to be most abundant in the half of the cell next the wall of the tubule, as may be seen in the transverse and longitudinal sections of tubules shown in Fig. 202, A, B, (× 700), In a few of the tubules in the pyramidal portions of the kidney the organisms had developed more abundantly, so that the cells were filled with a dense mass of micrococci such as may be seen in Fig. 203, A (× 700). In sections through blood-vessels also a moderate number of Bacteria were seen mixed with the blood-corpuscles.

Here then it is clear that we have again the kind of appearances which we have a right to expect if the microorganisms had developed in the epithelial cells of the kidney by heterogenesis. We have the cells full of particles developing into fully formed Bacteria, and, what is more important still, we have them within almost every epithelial cell to be seen in the sections.

From the point of view of the really absurd suggestion as to organisms being found in such an abdominal organ as the kidney, by reason of its "proximity to absorbing mucous membranes," it may be well to recall the fact that the sheep's kidney is encased in a mass of fat from half an inch to an inch in thickness, and that, as an additional barrier separating wandering microorganisms from the epithelial cells of the organ, there is still the thick and tough capsule which I have thought it not useless to show in Fig. 203, B (× 700). Let anyone compare M. C. Potter's description (p. 295) of the mode in which Bacteria are enabled to effect an entry into a single vegetable cell, and then let him imagine what an army of Bacteria would be needful, with all the cytase they could excrete, to get through such a tough and thick layer as that presented by the fibrous capsule of the kidney. But surely the whole notion as to such a mode of infection of the kidney and other abdominal organs is too absurd for serious consideration.

We are then driven back to inquire whether it is true that the blood is germless, and whether it has in reality the bactericidal qualities with which it is credited. I have no evidence whatever to oppose to these beliefs, nor is it easy to see, even if bacteriologists generally should be wrong in their views as to these points, how it would suffice to explain the development of Bacteria within all the cells of a kidney treated in the way I have mentioned.

It is perfectly certain that the blood of healthy persons does

not contain any appreciable number of active Bacteria. But are bacteriologists right in supposing that such Bacteria as get into the blood stream are "destroyed?" May they not rather be reduced to a condition of latent vitality? Their answer to this is, that if it were true, the organisms would be capable of revealing their presence when suitable media were inoculated with them and subsequently exposed to proper incubating temperatures. And it is the negative results of all such experiments with the blood of healthy animals that confirm them in their belief as to the germicidal qualities of the blood.

Nor, in fact, if the blood were assumed to be full of latent germs of Bacteria would it be easy, as I have intimated, to see how that would enable us to explain the development of Bacteria within almost every epithelial cell in the kidney referred to. Could organisms reduced to a condition of "latent vitality" penetrate the walls of the capillaries, and thence migrate into all the cells of a kidney tubule? The notion is again too preposterous to be entertained; so that we are compelled by evidence of a most convincing character to admit that the Bacteria have in reality been born in the individual cells of the kidney—we are compelled to believe that heterogenesis, has, in fact, been taking place here as in the other instances previously cited.[1]

XXIV. ON THE STATE OF THE QUESTION AS TO THE PRESENT OCCURRENCE OF ARCHEBIOSIS.

The *de novo* origin of Bacteria from specks of living matter born directly from certain mother liquids—that is, by a process of synthesis from its elements, and just as crystals are born in other mother liquids—is what I mean by *Archebiosis.*

My experiments many years ago in regard to this question did not convince the scientific world that there was decisive proof of the present occurrence of such a process. The last experiments I published, however, on this subject, together with the interpretation of their results given by others, were of such a nature as to convince me of the futility of further attempts in this direction. They tended to show the practical insolubility of the question by flask experiments with superheated fluids. For that reason I ceased to make and publish further experiments of this kind.

Anterior to the publication in 1876 of my experiments on the fertilisation of boiled acid urine by an accurately neutralising dosage with boiled liquor potassæ,[2] it had been generally inferred

[1] The importance from the point of view of medical science of this proof of the *de novo* origin of Bacteria I have explained in Appendix iii.

[2] *Proceed. of Royal Soc.* 1876, vol. xxv., p. 149, and *Journ. of Linn. Soc.* (Zool.), vol. xiv.

that the continued barrenness of urine and other acid fluids previously superheated was a proof that heat to the extent employed was adequate to kill any organisms or their germs pre-existing in such fluids.

This view was based upon the fact that living Bacteria or their germs seemed invariably to increase and multiply in suitable fluids placed under conditions favourable for fermentation.

It must be admitted, however, that this assumed proof was fallacious. Life may persist even when there is not sufficient vigour in the living things to enable them to grow or multiply in the previously heated medium in which they find themselves.

And as long as such inert or partially devitalised organisms remain in certain fluids, they may never regain sufficient vigour to enable them either to grow or to multiply. They may continue as if dead, though not actually dead.

But in other fluids of a different chemical constitution, or nutritive value, it is certainly possible that such partially devitalised organisms may become reinvigorated (especially if their revival be further aided by more stimulating conditions) so as to enable them again to grow and multiply. In this way it must be admitted as possible that life may appear in previously superheated fluids simply because organisms, hitherto assumed to be dead, have been made by the means adopted to show themselves as living.

Still the belief that the barrenness of a superheated fluid (favourable in nature and favourably placed for the promotion of fermentation) was due to the fact that all its contained organisms and their germs were killed—had been commonly accepted as true and the results of experiments of such an order had been cited as an important portion of the evidence and arguments tending to show, as some said, that "spontaneous generation" was a chimera. My experiments with acid urine and liquor potassæ, however, necessitated the rejection of this belief by those who had previously used it in the manner I have indicated. I was, in effect, promptly told that the organisms which all previous experimenters had spoken of as being killed in boiled acid urine could not have been killed. I had by subsequent neutralisation of the fluid, and by exposing it to a far higher incubating temperature (50° C.) than had been employed by previous experimenters, succeeded, they in effect said, in bringing to life organisms which every one else had supposed to have been killed. It was this, rather than Archebiosis, that accounted for the swarms of Bacteria within the experimental flasks.

Now the requirements, underlying all attempts to prove the "spontaneous generation" of living matter by flask experiments with superheated fluids, are of two kinds. We have (*a*) to

ascertain as far as possible, by preliminary trials, the lowest amount of heat, and the duration of its application, which is necessary for the destruction of pre-existing living things within the experimental vessels. And we have (*b*) to see whether it is possible that fluids submitted to the *lowest necessary amount of heat* to ensure the destruction of all pre-existing life can still, by subsequent treatment and favourable conditions, be induced to engender living matter.

Everything of course turns upon these words "lowest necessary amount of heat."

The destructive and deteriorating influence of heat upon the organic fluids with which experiments have to be made must be obvious to all; and the ultimate deteriorating results may not be very different where recourse is had to one process of heating at a high temperature, or to several at rather lower temperatures as in the "discontinuous process" adopted by Tyndall. No one need doubt the truth of the *naïve* remark of Fischer[1] when he says, "Infusions of organic substances, if they be only boiled long enough, will remain sterile for years."

The essential question is, therefore, what is the lowest necessary amount of heat to ensure, on the one hand, that all pre-existing living things shall be killed and to avoid as far as possible undue deterioration of the fluids employed. But it has now been thoroughly brought home to us how impossible it is to arrive at a trustworthy conclusion in regard to this point. In death point experiments (*a*) the barrenness of the fluid may merely mean that the organisms are inert rather than dead. I freely admit this as a possibilty, and moreover, judging from the past history of this question, I feel certain that even if there were a new basis of agreement in regard to the death point of micro-organisms in any particular fluid such as existed anterior to 1876 (when acid urine boiled for five minutes was universally said to be sterilised because its organisms were killed) that this common point of agreement would be immediately renounced by many directly some other experimenter claimed that from such a basis he had demonstrated the "spontaneous generation" of living matter. He would doubtless be told again that "spontaneous generation" was a chimera, and that he had only succeeded in resuscitating germs which all his opponents had hitherto believed to be dead. The proof of this being so would for them (as believers in the chimera assumption) lie in the fact that living things had appeared within the experimental vessels.[2]

[1] "The Structure and Functions of Bacteria," Trans., 1900, p. 51.

[2] As an instance of this tendency I may cite the fact that Burdon Sanderson after describing (*Brit. Med. Journ.*, February 13, 1875, p. 201) the preparation of his "pyrogenous liquid" and remarking that ordinary Bacteria are "inevit-

Such see-saw work might go on indefinitely. Conviction would probably never be brought about, because this ever recurring objection could not possibly be met, so long as the experimenter had to confine himself to sterilising degrees of heat which would allow him the least chance of success.

I have come to the conclusion, therefore, that this mode of experimentation with heat-degraded fluids in small vessels not only cannot be regarded as a fair test as to the possibility of the present occurrence of Archebiosis under more natural conditions, but that it is, moreover, one which is not likely ever to admit of the recognition that absolute proof has been afforded.

We must each of us form our own views as to the probability of the present occurrence of such a process, seeing that experimental evidence has been reduced to a virtual deadlock. Many will, doubtless, continue firmly to believe that "spontaneous generation" is a chimera; but others, even in the absence of absolute experimental proof, will prefer to believe in the continuity of natural phenomena. To affirm that living matter was formed once in the far remote past by natural agencies, but never again or now, is arrogantly to assert without knowledge that the uniformity of natural phenomena is at fault.

The first portion of a crystal begins to separate from its mother liquid in obedience to the same natural laws as those which determine its increase or growth; and if fragments of living matter also increase or grow through the sole agency of natural laws, is it logical or consistent for those who think so to assume that the first portions of such living matter cannot also separate from certain fluids under the influence of the same natural laws?

Yet some of the persons—believers in the philosophy of evolution—who allege their disbelief in the logical conclusion that living matter may originate as well as grow in suitable media by the concurrence of mere natural causes, rely, as one main reason for this disbelief, upon the statement that such a mode of origination is opposed to all antecedent experience. This they do under the influence of the dictum *omne vivum ex vivo*—a wide generalisation seemingly warranted by experience, but which may never-

ably killed by immersion in absolute alcohol," went on to say it is therefore "clear that the fever liquid contains no active Bacteria. It is equally clear that it contains that particulate material out of which Bacteria spring, for otherwise it could not be deprived of its fertility by filtration. It must, therefore, contain particles which, although they resist alcohol and heat yet are endowed with a latent capacity of development." He assumed the existence in this fluid of resting-spores of Bacteria capable of resisting the lethal influence of boiling alcohol, solely because he found that Bacteria would appear in this fluid. No independent proof of the existence of Bacteria germs or spores capable of resisting boiling alcohol was deemed necessary.

theless be fallacious; because what might contradict it lies outside and of necessity altogether beyond the range of their experience. Those who use this argument, in fact, forget that, from its very nature, the process in question (Archebiosis), to whatever extent it may have occurred, must always have remained beyond the range of human experience. Nobody with unaided eyes could ever have witnessed the birth from fluids of invisible particles, by which the "spontaneous generation" of living matter must always commence, if it commences at all. And even when aided by the most powerful microscope nobody could decide when *minimum visible* particles appear in the field of view that such particles have proceeded from invisible germs rather than from a primordial synthesis of living units.

The tendency with Pasteur, and with Tyndall and others has been too much to ignore this latter possibility, to regard it as a chimera, and one not to be seriously considered. But this, as was well said by Rücker in his Presidential Address before the British Association in 1901, concerning the one-sided attitude of some in reference to another fundamental physical problem, is "*to beg the whole question at issue; to decide the cause before it has been heard.*"

Again, Chamberland, a previous assistant of Pasteur, in a thesis published in 1879[1] tests the vital resistance of Bacilli spores by heating them in *neutral* infusions, which may be capable of *originating* as well as of favouring the growth and multiplication of such bodies. He concludes that a heating for five hours at 100° C. is needful for their destruction in some of these fluids.

But many years ago, after what I considered to be some very extravagant statements by Tyndall concerning the powers of resisting heat possessed by, what were for him, hypothetical germs contained in "old hay," I made a long series of experiments with multitudes of actual hay germs (that is, with spores of the hay Bacillus) which had been allowed to dry in their mother liquid on glass slips five years previously. I tested the death point of these old Bacilli spores in acid urine that had been boiled, in which I knew that Bacilli or any such organisms did not originate; though I had ascertained that when they had not been previously heated the inoculation of boiled urine with these spores and the dried media in which they were contained would cause such organisms to grow and multiply therein most freely. But when an emulsion of the old spores was made (by scraping the dried scum from the glass slips into a small quantity of water) and drops of this emulsion were added to some acid urine, and this inoculated fluid was subsequently boiled for twenty minutes, the organisms invariably *seemed* to be killed—as the fluids remained unchanged.

[1] "Recherches sur l'origine et le dévelopement des organismes microscopiques." *Thèse*, No. 420. (Faculté des Sciences, Paris).

Thus boiling for twenty minutes in a slightly acid fluid seems to destroy the spores, while boiling for five hours is said to be necessary in another neutral fluid (hay infusion) to ensure the destruction of similar spores. This, of course, may be true, or it may not. Spores actually existing in the hay infusion may be much more speedily killed; and it may be the power of giving birth to organisms (the germinality of the fluid) which requires the five hours at 100° C. for its extinction.

It is true that there is good evidence (such as Chamberland advances, and of a kind to which I had long ago called attention) to show that growth and multiplication of Bacilli does not take place so freely or quickly in acid as in neutral solutions. But then might not, even ought not, the same difference to hold good in regard to the possible origin of living units in the respective fluids? The conditions less favourable for growth should be less favourable for origin, and *vice versâ*.

The difference between twenty minutes and five hours of exposure to 100° C. is enormous; but the difference between the rate of growth and multiplication of Bacilli in slightly acid and in neutral fluids respectively, however real, is comparatively slight.

Hence, the question of the possible germinality of the hay infusion cannot be ignored. But here again, as ever, we cannot completely get rid of the doubt, that in my death-point experiments the spores were not really killed by the twenty minutes' boiling in the acid urine. Chamberland might retort that they may have been merely rendered inert by the heat so long as they remained in this fluid. To attempt to get rid of this difficulty is to come face to face with its correlative, namely, how are we to decide, when dealing with other more favourable fluids, whether an appearance of organisms therein is to be ascribed to *survival of germs*, or to *germinality of the fluids*.

It is worthy of note, however, that my opponents in this question have invariably assumed, rather than proved, various points needful to give adequate warranty to their interpretation of the appearance of living organisms in critical flask experiments.

They know quite well that spores are distinctly more resistant to heat than the parent organisms; they know that the parent organisms without spores are almost infinitely more common than such organisms with spores; they know that even under most favourable conditions such spores will often refuse to develop; yet whenever living organisms appear in the guarded fluids which have been heated far more than is necessary for the destruction of the parent organisms, they invariably assume that the much rarer spores have been present, and they further find it necessary to assume that the spores which are often most slow to develop under favourable conditions, now, in spite of the injurious

heating and unfavourable conditions in which they are placed, straightway hasten to develop, grow and multiply. This is, surely, more like begging the question at issue, than judging in accordance with evidence.

In addition to the above considerations, it may not be without interest to some that attention should be called to the following facts, seeing that they may help to show in which direction the balance of evidence lies.

(*a*) Thermal Death Points and their Relation to certain Old Flask Experiments.

The dicta at present given in regard to thermal death points are these. Pasteur formerly said that a brief exposure to 110° C. was sufficient to sterilise all fluids; but now his former assistant, Chamberland, as a result of investigations made between 1877 and 1879, and recorded in the thesis above referred to, has been induced to put the temperature rather higher. He says, however, that a temperature of 115° C. is sufficient to sterilise all fluids "completely and very rapidly," and therefore to kill any Bacilli spores that may be contained therein.

No statements as to the lowest temperature at which we may be certain that the spores of Bacilli are killed are made by Fischer; he says,[1] however, that superheated steam in an autoclave "kills the toughest spores in one minute at 140° C." Macfayden also says,[2] "No spore, however resistant, remains alive after one minute's exposure to 140° C."

In reference to the spores of Fungi, de Barry[3] mentions that in their dry state 130° C. has been necessary for their destruction, but he adds "the death point of the spores of Fungi is often much lower than this in water or watery vapour, and it has not been shown that any can under these circumstances survive a temperature of 100° C."

I have, however, recorded experiments in which organisms have been plentifully found in fluids that had been heated within closed vessels to higher points than any of those above mentioned.

Thus, experiments were made with neutral and faintly alkaline *hay* infusions of this nature.[4] "In each case about half an ounce of the fluid was used, half filling a tube which was sealed when cold; so that above the fluid there was ordinary air . . . . In twenty-one cases they were heated to 248° F. (120° C.) for thirty minutes; and in five of these latter trials (all with the same hay infusion) no fermentation subsequently occurred. In the other instances more or less distinct fermentation supervened—though in some the signs of change before opening the vessels were only slight.[5] . . . Also in five other experiments in which *milk* was heated to 240° F. (115·5° C.) for ten minutes, fermentation more or less marked occurred in each case in from two to ten days."

The nature of the changes seen in the fluids and the kinds of organisms

[1] "The Structure and Functions of Bacteria," Trans., 1900, p. 76.

[2] *Nature*, February 7, 1901, p. 361.

[3] "Fungi, Mycetozoa and Bacteria," p. 347.

[4] See a Memoir "On the Conditions Favouring Fermentation," *Journ. of Linn. Soc.* (*Zool.*), vol. xiv., pp. 50-53.

[5] These tubes on account of their subsequent treatment were necessarily of very thick glass, and were thoroughly *flambé* when I prepared them. shortly before they were made use of.

found therein are fully described in this memoir in subsequent pages (pp. 53-60), and illustrations of some of the organisms met with are given.

Two points worthy of note concerning the changes occurring in such hay infusions are these. When an infusion of this kind has been heated only to 100° C., and subsequently becomes fertile, it may always be seen to change uniformly throughout its whole bulk. There is no cloudiness gradually spreading from centres of infection; but a uniform opalescence, gradually increasing to actual turbidity, throughout the fluid, which at the same time becomes gradually paler in colour. On the other hand, where this or any other clear infusion has been heated to 110° C. and upwards, such a change almost never occurs. We have then altogether different appearances. The bulk of the fluid remains clear, though, after days or weeks, a very slow accumulation of sedimentary matter occurs; and in this, on microscopical examination, in addition to different kinds of Bacteria it is most common to find Torulæ and other Fungus germs together with some Mycelia, such as I have figured as found in the experiments above referred to.

The finding, however, of Torulæ, and other Fungus germs, with Mycelia, in these superheated fluids, is a fact of great significance in view of the statement by de Barry (with whom others agree) in regard to spores of Fungi generally—namely, that they have never been shown to be capable of surviving in fluids at a temperature of 100° C.

This, however, is not all; I can point to two experiments in which other fluids were heated in closed tubes to 132-135° C. for twenty minutes, and to one in which the fluid was heated to 144° C. for five minutes, and yet after some weeks, when the flasks were opened Torulæ and other Fungus spores were found among the sedimentary matter, such as I have elsewhere described and figured.[1] I can with the utmost confidence refer to these three experiments as being free from all flaw or ambiguity. The fluids were heated, for the times and degrees mentioned, in an autoclave, and when the flasks were opened very various living organisms were found in the fluids. Other experiments might be cited, but those referred to are sufficiently typical.

(*b*) The Production of Different Microorganisms at Will from Healthy Urine.

One of the arguments of Pasteur, and also of Lister and others, against the present occurrence of Archebiosis was this. They met the objection that in flask experiments with suitable fluids negative results are due to the degradation of the organic constituents in the fluids, owing to the heating of them many times to minor degrees or once to some major amount, by saying that blood or urine if taken directly from the body, with all necessary precautions against external contamination, can be preserved indefinitely without change and without the appearance therein of any microorganisms. This, of course, is what ought to occur if such fluids are, as they maintained, germless, and if the present occurrence of Archebiosis is such a chimera as they imagined it to be.

Twenty years ago I resolved to test these statements for myself, taking urine as the test fluid because of the greater facility for multiplication of experiments therewith. During a period of three months I made nearly two hundred experiments, using all the precautions recommended by Lister as needful in such experiments. The urine was passed either into one of his receivers or else directly into the experimental vessels, and in all cases the vessels employed were thoroughly *flambés*. The urine experimented with had only a moderate amount of acidity, as it was always found to be capable of being neutralised by from 7-10 minims of liquor potassæ to the ounce.

[1] See "The Beginnings of Life," vol. i., pp. 441, 443 and 447. During the process of raising the temperature to 132-135° C. and subsequent cooling, these fluids would have been exposed to at least 115° C. for nearly one hour, and of course longer still in the case of the fluid heated to 144° C.

The results that I obtained in the large majority of cases were divisible into four categories: —

(1) Urine passed into a flambé vessel, and subsequently kept at temperatures ranging between 16° and 30° C. would, as Pasteur, Lister and others say, remain clear and free from all signs of change.

(2) Urine passed into a flambé vessel and subsequently kept at a temperature of about 45° C. would almost invariably become turbid within three days, and on examination be found to swarm with Micrococci.

(3) Urine passed into a flambé vessel which had been fully or two-thirds neutralised with liquid potassæ, and subsequently exposed to temperatures ranging from 45° to 50° C., would generally become turbid in seventeen to twenty-four hours, and on examination be found to swarm with Bacilli only.

(4) The addition of lesser amounts of liquor potassæ, that is, sufficient for half neutralisation, or a little less or more, would generally lead to a mixture of Micrococci, Staphylococci, and Bacilli.

I found I could thus obtain, almost at will, urine containing Micrococci only, Bacilli only, or mixtures of these two organisms.

In all the experiments where liquor potassæ was used, I had previously heated it in tubes to 105° to 120° C. for one hour, as I had formerly done to meet objections made by Pasteur in regard to other experiments.

I have little doubt that blood or milk taken from an animal with all the precautions possible, and subjected to such temperatures as I have employed would speedily show signs of change. I have, however, made no experiments with either of these fluids. Were I to do so, and obtain positive results I should doubtless be told that I had not guarded against this or that source of error—which would, in fact, be difficult enough in the case of the latter fluid, with which no one as yet has succeeded in obtaining uniform results.

c) The Changes induced in some Organic Fluids by their Passage through Chamberland and Berkefeld Filters.

Later still, when Berkefeld and Chamberland filters came into use, it was said that the filtration through porcelain, with the latter filter especially, was so effectual that it would stop the passage of all Bacterial germs, and that organic infusions so treated, even when they had undergone no degradation by heat, would remain unaltered and show no signs of fermentation.

If true, this seemed to me at the time the strongest evidence that had yet been brought forward against the present occurrence of Archebiosis, so I resolved to make some experiments myself with hay infusions prepared in the ordinary way, and as I have described on p. 66. This was done, and I found it quite true that a hay infusion passed through a Chamberland filter would subsequently remain for long periods quite clear, even when kept at a temperature of about 93 F°. (34° C.), though I observed that a very slight deposit after a few weeks was apt to accumulate at the bottom of the vessel.

It occurred to me then that the passage through the extremely minute interstices of such a filter might have altogether altered the constitution of the fluid, in a different way, it is true, but with the resulting production of changes therein very analogous to those caused by superheating.

I therefore made some experiments in order to test this supposition, and will quote one of the most decisive of them.

On October 20, 1900, a hay infusion was made by macerating two drachms of finely cut hay in ten ounces of tap water for four hours at about 82° F. (28° C.), and the fluid was subsequently divided into two equal portions.

(*a*) Was simply passed through one layer of Swedish filtering paper.

(*b*) Was passed through a Chamberland filter.

Each fluid was placed in a small lipped beaker to which one drachm of tap water was added; each beaker was then covered with a circle of glass

and the two were left side by side on an incubator at a temperature of about 65° F. (18° C.)

In twenty hours the fluid in (*a*) was found very opalescent throughout; that of (*b*) was still clear.

October 23. (*a*) Very turbid, and with a thin pellicle on its surface. (*b*) Still quite clear.

October 25. (*a*) Very turbid, and covered with a pellicle containing *Zooglœa* areas; no Ciliates seen, but Monads were very abundant. (*b*) Fluid still quite clear.

October 27. (*b*) Fluid still quite clear. It was now inoculated with a small portion of the fluid and pellicle of (*a*).

October 29. (*b*) Fluid still quite clear.

November 1. (*b*) The freely inoculated fluid still remained quite bright and clear at the expiration of five days.

Nothing could show more clearly than this how greatly the hay infusion must have been altered in its constitution by its passage through the Chamberland filter. As I imagined, changes were undoubtedly produced which led to its behaving much as a hay infusion heated to 115° and 120° C. would do. I have often found that such fluids, when removed from experimental vessels, are also very little prone to undergo change, even when freely exposed to air and what it may contain. Moreover, there is another striking resemblance. In the sediment which collects at the bottom of a vessel containing a hay infusion that has been passed through a Chamberland filter, I have, after several weeks, found Torulæ and other Fungus spores, together with small Mycelia, though the supernatant fluid was still perfectly clear. And, as I have shown, the same kind of change is apt to occur in a superheated hay infusion. When either of these fluids is subsequently exposed to the air, a tuft or two of Mould may after a time appear upon its surface; but as a rule, in neither case do the fluids become turbid with Bacteria—at all events within a week or ten days.

Just after I had made these experiments, No. 438 of the *Proceedings of the Royal Society* was received in which I found a communication by Macfadyen, Morris and Rowland "On Expressed Yeast-cell Plasma." They tested the effect of the filtration of this juice through Berkefeld and Chamberland filters, and they found that such "filtration decreases to a considerable extent, but without entirely destroying both the auto-fermentation and the action of the juice on sugar"; they found also that the specific gravity of the filtrate was most notably lowered.[1] Again, when speaking of kieselguhr, a very fine diatomaceous earth, which in a compressed state forms the basis of the Berkefeld filter, they say (p. 253): "that kieselguhr has the power of arresting the passage of certain albuminous bodies can easily be demonstrated. Thus we found that egg globulins are almost entirely retained in a kieselguhr sponge, and even albumin and serum proteids are retained to a certain extent."

Thus, removal from an organic solution of some of the larger colloid molecules by filtration, seems to have an effect upon the fluid not very different from that which is produced by the probable breaking up of such molecules during a process of superheating. The fluid in either case is notably degraded, and what I have termed its "germinality" is proportionately lowered. I am, therefore, no longer surprised at the comparative stability of an unheated hay infusion which has been passed through a porcelain filter.

Before leaving this subject, it may be of interest to many to call attention to the singularly close analogy existing between the

[1] The observations were principally made with a Berkefield filter, which is known to pass molecules that would be stopped by a Chamberland filter.

problems concerning the birth of crystals from mother liquids and the possible birth of specks of living matter from fluids of another kind.

This has been most ably brought out by Prof. Léo Errera in an essay entitled "*A propos de génération spontanée*,"[1] an abstract of a portion of which I subjoin.

The Origin of Crystals.

Errera points out that in 1865 Violette and Gernez demonstrated, independently, that crystallisation was brought about at ordinary temperatures in supersaturated solutions of sodium sulphate by the access thereto of microscopic crystals of this salt, which exist (almost always) as constituents of atmospheric dust. These microscopic crystals, not much larger than the germs of microbes, act much like germs, and lead to the separation of crystals from the mother liquor.

Ostwold showed that salol, a white crystalline substance which melts at 39·5° C., may, when in this condition, be rubbed with a hair, a thread of glass or of platinum, or any angular substance, without inducing crystallisation. It will remain in the fluid state so long as it is not brought into contact with a crystal of salol; but directly it is touched with one of the previous objects after it has been brought into contact with salol crystallisation is at once initiated in the fluid, and spreads rapidly through its mass. The inoculating object, however, may be easily sterilised by raising it even momentarily to some point above 39·5° C. Very similar results were obtained with supersaturated solutions of hyposulphite of soda and chlorate of soda, which remained without change (sterile) until they were inoculated with microscopic crystals of these substances, of extreme minuteness.

In face of such facts, says Errera, "it is natural to ask how the first crystal of each of these substances is born." And the reply, he says, must be "by *spontaneous generation*."

Some supersaturated solutions may (when protected from the advent of all germs) be rendered fertile by allowing them to evaporate and become more concentrated, others by lowering the temperature of the solutions, and others still, even by slight mechanical shocks when the supersaturation is strong. De Coppet also made out the interesting fact that "other things being equal, the first crystal forms more quickly in large than in small masses of liquid" dealt with in either of these ways.

In illustration of the effects of temperature some important investigations have been made with *bétol* by Tammann, of Dorpat. "Its point of fusion is more elevated than that of salol: it is 96° C. Melted at about 100°, and subsequently kept in little sealed tubes and cooled, it remains liquid for a period more or less long. But sooner or later, according to the temperature and the quantity of liquid employed, centres of crystallisation gradually appear, whence solidification is slowly diffused throughout the whole mass. For the same volume of liquid the number of its centres of crystallisation increases with the cooling, attains a maximum, and then decreases pretty quickly. In the case of bétol the most favourable temperature—what in biological language would be called the 'optimum temperature' for the spontaneous generation of crystals —is about 10° C. But above and below this the phenomena progressively diminish, so that above 25°, or below—5° the bétol can be kept for a long time in the liquid state. . . . . In addition, Tammann discovered that small quantities of foreign bodies, soluble or even insoluble (such as rock crystal or glass) suffice greatly to modify the number of germs—sometimes increasing and sometimes diminishing them."

[1] *Revue de l'Université de Bruxelles*, t. v., 1899-1900, Mai.

Now comes a statement which is rather surprising: "But the temperatures most favourable for the generation of crystals are not the most favourable for their rapid growth; the optimum temperature for growth is notably higher than that for generation." Thus, expose tubes containing liquid bétol to about 10° for several minutes, and the fluid will still appear perfectly limpid, the newly-formed crystalline germs being so very minute as to be invisible. Now, as though dealing with a culture of microbes, expose these same tubes to an incubating temperature of about 70°, and in a few moments it will be seen that the crystalline germs have increased sufficiently to show themselves through the whole mass of the liquid." But it is important to note that if the tubes are exposed to this high temperature "without having been previously exposed to the low temperature favourable to the birth of germs, no crystallisation of bétol shows itself, even after a long trial."

The exact conditions under which, in liquid salol, a spontaneous generation of crystals becomes possible is unknown, although it is well known that crystallisation may be immediately induced in such a liquid by inoculating it with microscopic crystals of this substance at some temperature below 39·5° C. It is almost the same with glycerine. Up to 1867 this substance was only known in the fluid state, but then, after exposure to a low temperature and mechanical shocks, during a long railway journey from Vienna to England, a quantity of it was found to have become converted into a mass of white acicular crystals. This change to the crystalline state has been observed on a very few occasions since, but the conditions have not yet been accurately ascertained. It cannot be induced at will, though it can always be set up at once when the liquid glycerine is inoculated with a crystal of that substance; and, as with bétol, the process is rendered more rapid by heat up to a very moderate point. The heating must, however, only be slight, because at about 18° C. all crystals disappear, and the fluid would be sterilised.

Thus, from the point of view of crystallisation, there are two classes of fluids; one (*a*) in which, under appropriate conditions, there may be a spontaneous generation of crystals, as well as a spread of the process induced by inoculation; and another (*b*), where the latter process only seems possible and in which generation has never been known to occur. These two classes of fluids, of another order, have, of course, been long recognised also by those who believe in the "spontaneous generation" of microbes.

What is said above as to the importance of the existence of particular conditions for the origin of crystals, will suffice to emphasize what I have dwelt upon as to the even greater importance of suitable and favourable conditions for the production of the higher and more subtle molecular combinations that are to lead, in fluids of another kind, to the birth of invisible units of living matter. We may, of course, each of us form our own opinion as to the direction to which the balance of evidence inclines concerning the present occurrence of this latter process. And if, admitting that absolute and rigorous proof of the present occurrence of Archebiosis cannot, for the reasons previously stated, be given by means of experiment, we may on general grounds, as believers in evolution, in the physical doctrines of life, and in the uniformity of natural phenomena, safely recognise the high probability that it is a process which still occurs, and ever has been taking place in suitable media since the time when living matter made its first appearance on the cooling surface of our planet.

Is it logical and consistent, or is it the reverse, to suppose that natural life-producing causes were operating upon some limited portion of the earth's surface for some limited time, and that such causes have ever since ceased to be operative?

Little is said upon this subject by my critics. Those of them who are evolutionists are nevertheless in this position: their opposition to a belief in the continuance of Archebiosis requires them to postulate an inexplicable departure from the continuity of natural phenomena. But is there any reason to believe that the transmission of light and of sound do not go on now as ever? Or, again, that chemical affinities are not of the same kind now as they have ever been among the various so-called elements entering into the composition of the visible universe. It is, of course, a mere truism to say that a belief in the continuity of natural phenomena is a cardinal tenet of scientific faith, and one which is taken for granted in all scientific reasoning.

Yet a disbelief in the occurrence of Archebiosis here and now on the surface of our earth by those who assume it to have occurred in the past would be such a notable departure from this otherwise firmly accepted article of scientific faith, that one can understand the reluctance of so distinguished a physicist as Lord Kelvin to give his assent thereto. He seemed to prefer to evade the problem, so far as this earth is concerned, by suggesting that Life may have come to us on a "moss-grown fragment from the ruins of another world."[1] Nothing was said by him as to the origin of the life on this other world — whether living matter had been engendered there by natural causes, or whether it had similarly come to that world from some other source. His suggestion may be said to be elusive rather than luminous; though a belief in the possibility indicated by him permitted its author's faith in the continuity of natural phenomena to remain perhaps undisturbed.

As I have already pointed out, absolutely no reason exists for a disbelief in the present occurrence of Archebiosis apart from the fact that in spite of numerous attempts to prove its occurrence, no one has yet succeeded in convincing the scientific world that it does occur. There is no vestige of evidence to show that under favourable conditions the process may not be continually taking place around us. The birth of invisible particles of living matter in fluids would not oppose or contradict the actual experience of anyone. Yet this, and this only, is what is meant by Archebiosis.

[1] Inaugural Address to the British Association, *Nature*, Aug. 3, 1871, p. 269.

XXV. ON THE RELATION OF HETEROGENESIS TO ALLOTROPISM AND ISOMERISM; AND ON THEORETICAL OBJECTIONS TO PER SALTUM DEVELOPMENT.

In his extremely interesting "*Studies in the Theory of Descent*"[1] Prof. Weismann has a concluding essay on "The Mechanical Conception of Nature," in which, as a consistent evolutionist, he argues powerfully in favour of purely physico-chemical doctrines of life, and against the existence of any special "phyletic vital force," or "internal principle of development" as von Hartmann terms it. Incidentally he refers to and discusses (pp. 697-708) the possibility of Heterogenesis or *per saltum* development in a fuller manner than I have met with elsewhere, and comes very positively to the conclusion that it is an untenable doctrine.

As I am fully in accord with him in regard to the main purpose of this essay, I venture to attempt to show that, in accordance with his own principles, a belief in Heterogenesis, especially among lower organisms, far from being untenable, as he supposes, is, in fact, rather a logical corollary from our general physico-chemical knowledge.

Weismann says (p. 717):—"We are still far removed from completely understanding the mechanism by means of which the organic world is evoked—we still find ourselves at the very beginning of knowledge. We are, however, already convinced that both the organic and the inorganic worlds are dependent only upon mechanical forces, for to this conclusion we are led, not only by the results of investigators who have restricted themselves to limited provinces, but also by the most general considerations. . . . . for the naturalist the mechanical conception of nature is the only one possible." He concludes, however, with the following words: "The final and main result of this essay will thus be found in the attempted demonstration that the mechanical conception of Nature very well admits of being united with a teleological conception of the Universe."

It should, I think, be clear, though I see no reference made to the subject by Weismann or others, that among the lower forms of life whose mode of increase is by fission,—or mere "discontinuous growth" as Huxley termed it—we need make no appeal to heredity. The forms of these comparatively simple living things —whether of vegetal, of animal, or of indeterminate type— must be regarded as natural products resulting from their molecular constitution and the influence of environing forces, just in the same way that the forms of crystals are the results of their molecular constitution under the influence of their particular media. The forms of crystals are regarded as in the main due

[1] Translated and edited by R. Meldola, with a Preface by Darwin. vols. ii., 1882.

to the polarity of their molecules; and similarly the forms of all simplest organisms must be due in the main to the polarity of their molecules—"organic polarity," as Herbert Spencer has termed it. In both cases, of course, external conditions exercise a modifying influence.

So long as external conditions remain the same the crystal preserves its form; and, similarly, under like external conditions, the organism preserves its form, and when it multiplies by fission it continues, to this extent, to "breed true." It cannot be said by Weismann and his school that such comparisons, and others on which I am about to dwell, are unwarrantable. He himself says (p. 674): "The living organism has already been often compared with a crystal, and the comparison is, *mutatis mutandis*, justifiable. As in the growing crystal the single molecules cannot become joined together at pleasure, but only in a fixed manner, so are the parts of an organism governed in their respective distribution. . . . In neither instance do we know the final causes which always lead to a given state of equilibrium; in the case of a crystal it has not occurred to anybody to ascribe the harmonious disposition of the parts to a teleological power; why then should we assume such a force in the organism? . . . Variability and heredity, as well as correlation, admit of being conceived as purely mechanical; and must be thus regarded so long as no more cogent reasons can be adduced for believing that some force other than physico-chemical lies concealed therein."

In not-living matter we have combinations of molecules of all degrees of complexity—beginning with those entering into the composition of the simple elements, and ending with the big and highly complex components of various colloids. And seeing that the so-called elements are now supposed to be built up by molecules composed of different numbers of similar atoms, the difference between elements and compounds simply depends upon the likeness or unlikeness of the atoms entering into the composition of their molecules.

Thus molecular composition is an important item even with reference to substances that are looked upon as elementary—different modes of composition or arrangement of the atoms of like kind sufficing to produce what are known as "allotropic" states. We are most familiar with these as they are presented to us in the various forms of carbon. The differences between the diamond, graphite, and pure charcoal are most striking, and yet these are all different states of one and the same elementary substance. Oxygen, sulphur and phosphorus, as well as arsenic, antimony and other metals also exist in allotropic states in which they exhibit properties wholly different from those of their common forms. It will be easily understood, therefore, that in compound substances a greater and greater possibility of mole-

cular rearrangement arises in proportion to their atomic complexity. Gradually, in fact, this atomic complexity becomes the all-important character of a compound, and one to which the nature of the constituent atoms is altogether subordinate. In proof of this one has only to refer to the multitudes of "isomeric" compounds having wholly different properties made up of carbon, hydrogen, and oxygen in the same relative proportions, and to the enormous number of other isomeric compounds resulting from the varying modes of arrangement of a few definite elements.

The different states in question—that is, the transformations—are brought about under the influence of ordinary physical conditions: changes in temperature for the most part, and more rarely changes in the incidence of light.

In the case of phosphorus its different states are as remarkable as those of carbon. Two of them are known respectively as "Normal" and "Red Phosphorus." The first variety is much more poisonous than the second; it is also colourless, crystallisable in rhomboid dodecahedra, soluble in sulphide of carbon, easily oxidisable, phosphorescent, and inflammable at a low temperature. The second form is scarlet red, amorphous, much less soluble, non-phosphorescent, and only inflammable at high temperatures. And yet such extraordinary differences are due only to an altered arrangement of the atoms in the molecules of phosphorus.

Crystallisable substances, moreover, are well known to assume different forms of statical equilibrium under the influence of changes in external conditions. Thus, referring to the article "Dimorphism" in Watts' "Dictionary of Chemistry" we find the following statements concerning changes in crystalline form which seem to depend principally upon temperature: "Many substances both simple and compound, crystallise in forms which belong to two or three different systems of crystallisation, or which, even if they belong to the same system yet exhibit such differences in their corresponding angles as to render it quite impossible to reduce them to the same form: this was first shown by Mitscherlich, in 1823. Such bodies are said to be dimorphous and trimorphous. The difference of crystalline form which they exhibit is associated with difference of specific gravity, hardness, colour, and other properties."

Again we have the following remarkable statements concerning certain definite substances. "Crystals formed at one particular temperature, and then exposed to that temperature at which crystals of a different kind are produced, often lose their transparency, and *without alteration of external form, become changed into an aggregate of small crystals of the latter kind;* examples of this alteration of structure are afforded by sulphur.

carbonate of calcium, mercuric iodide, and many other bodies. . . . *Mercuric iodide* separates from solution, and likewise sublimes at a very gentle heat, in scarlet tables belonging to the dimetric system; but, when sublimed at a higher temperature in sulphur-yellow, rhombic tables of the monoclinic system. The red crystals turn yellow when heated, and resume their red tint on cooling. The yellow crystals obtained by sublimation [at the higher temperature] retain their colour when cooled; but, on the slightest rubbing or stirring with a pointed instrument, the part which is touched turns scarlet, and *this change of colour extends with a slight motion, as if the mass were alive, throughout the whole group of crystals as far as they adhere together.*"

I have before me now a sheet of paper having a large circular area smeared over with a scarlet patch composed of the double iodides of mercury and copper; if I hold it over the lamp almost immediately the scarlet patch assumes a brownish-black colour; if the exposure has been only momentary, the patch almost immediately, on withdrawal, again assumes the scarlet colour; if I allow it to remain exposed to the heat for five seconds it takes about fifteen seconds for the brownish-black colour to disappear and for the scarlet colour to be restored. These changes will recur over and over again invariably, and just as they did more than thirty years ago when the specimen first came into my possession.

There can then be no reasonable doubt that the form of the crystal is a resultant necessity, predetermined by the molecular properties of the matter composing it, and the sum total of conditions acting thereupon at the time of collocation.

Similarly, there are excellent reasons for believing that the form and structure possessed by each organism is that which is necessitated by the nature and properties of the complex organic molecules of which it is composed. But important differences here present themselves by reason of the extreme complexity of the molecules of matter entering into the composition of living things. Various *protein* compounds constitute the all-essential constituents—compounds containing carbon, hydrogen, oxygen, and nitrogen, together with small proportions of sulphur and phosphorus by means of which the other elements are linked together in high multiples. These protein molecules, according to Frankland, are capable of assuming hundreds of different isomeric forms; and referring to this subject, Herbert Spencer says:[1] "it might be argued that these large aggregate atoms which constitute organic substance, are mechanically weak, are less able than simpler atoms to bear, without alterations, the

[1] "Principles of Biology," Revised Edition, 1898, vol. i., p. 14.

forces falling on them. That very massiveness which renders them less mobile, enables the physical forces acting on them more readily to change the relative positions of their component atoms; and so to produce what we know as rearrangements and decompositions."

Referring to the ultimate constituents of living matter as "physiological units," or in other words as special combinations of special organic molecules, Herbert Spencer writes as follows:[1] "We must infer that a plant or animal of any species, is made up of special units, in all of which there dwells the intrinsic aptitude to aggregate into the form of that species; just as in the atoms of a salt there dwells the intrinsic aptitute to crystallise in a particular way. It seems difficult to conceive that this can be so; but we must see that it *is* so. Groups of units taken from an organism (providing they are of a certain bulk and not much differentiated into special structures) *have* this power of rearranging themselves; and we are thus compelled to recognise the tendency to assume the specific form, as inherent in all parts of the organism. Manifestly, too, if we are thus to interpret the reproduction of an organism from one of its amorphous fragments, we must thus interpret the reproduction of any minor portion of an organism by the remainder. When in place of its lost claw, a lobster puts forth from the same spot a cellular mass, which, while increasing in bulk, assumes the form and structure of the original claw, we can have no hesitation in ascribing this result to a play of forces like that which moulds the materials contained in a piece of Begonia-leaf into the shape of a young Begonia."

As to the nature of the units which possess the property of arranging themselves into the special structures of the organisms to which they belong, a property which, for brevity, he speaks of as "organic polarity"—the same authority says[2] if this property "can be possessed neither by the chemical units nor the morphological units, we must conceive it as possessed by certain intermediate units, which we may term *physiological*. There seems no alternative but to suppose, that the chemical units combine into units immensely more complex than themselves, complex as they are; and that in each organism the physiological units produced by this further compounding of highly compound atoms have a more or less distinctive character. We must conclude that in each case some slight difference in the composition of these units, leading to some slight difference in their mutual play of forces, produces a difference in the form which the aggregate of them assumes."

The fundamental difference between a crystal and an organism

[1] *Loc. cit.*, p. 224. [2] *Loc. cit.*, p. 226.

lies, indeed, in the fact that the one is a *statical* and the other is a *dynamical* aggregate; this difference being dependent upon the great complexity of the molecules of which the latter is composed. Organisms are dynamical aggregates because among their molecules new motions and new arrangements are continually being assumed, in the course of which, in lower organisms, there frequently arises a spontaneous division of the parent mass—that is to say, fission or gemmation takes place.

The intimate nature of the process of reproduction characterising living things is revealed by a consideration of these processes of "fission" and "gemmation." All parts of very low organisms when thus separated from the parent have the power of developing into living things of a similar kind. This property has points of resemblance to the process whereby a fragment *broken* from a pre-existing crystal, and thrown into a suitable solution, gradually grows into a perfect crystal similar to that from which it has been derived.

What takes place in the true reproductive processes occurring among higher forms of life seems to be only a more complex exemplification of similar phenomena. As Herbert Spencer says,[1] "The assumption to which we seem driven by the *ensemble* of the evidence is, that sperm-cells and germ-cells are essentially nothing more than vehicles in which are contained small groups of the physiological units in a fit state for obeying their proclivity towards the structural arrangement of the species they belong to . . . . Thus, the phenomena of Heredity are seen to assimilate with other phenomena . . . . We must conclude that the likeness of any organism to either parent is conveyed by the special tendencies of the physiological units derived from that parent."

The view taken by Weismann concerning Heredity, as expressed in the essay now under consideration, was essentially similar. The power of organisms to transmit their properties to their offspring, he said, appeared to him "to be only conceivable," if we suppose "that the germ of the organism by its chemico-physical composition together with its molecular structure, has communicated to it a fixed direction of development—the same direction of development as that originally possessed by the parental organism."[2]

Looked at in this general sense the phenomena of heredity seem to be only special manifestations of the property above spoken of as "organic polarity," as a result of which, as we have seen, parts of lower organisms are capable under suitable conditions of reproducing similar entire organisms, just as a part of a crystal immersed in its mother liquid will, when the condi-

[1] *Loc. cit.*, p. 317. [2] *Loc. cit.*, p. 669.

tions are suitable, reproduce the form of the crystal from which it was derived. It is quite unnecessary here to follow Prof. Weismann into the very special views which he holds concerning the "continuity of the germ plasm," the absolute separation in higher organisms between their somatic and their reproductive cells, and the way in which he thus complicates the doctrine of Heredity—partly because I am concerned here principally with lower organisms multiplying in the main by mere processes of "discontinuous growth," and therefore independently of heredity except in the very general sense indicated by "organic polarity"; and partly also because it seems to me Herbert Spencer has shown that some of these hypotheses of Weismann are directly contradicted by many facts, and that the distinctions he draws are to a great extent merely fanciful.[1]

In a broad general sense, however, there has been so far community of doctrine, between these two philosophers. The differences between them, though great and important, are, after all, differences in detail, the significance of which does not affect my present argument. I am concerned with the community of their doctrines to the extent I have indicated, and now desire to draw attention to certain consequences dependent thereupon.

Neither Herbert Spencer nor Weismann allude to the existence of transformations in organisms at all comparable with the allotropic and isomeric transformations which are so remarkable in simpler forms of matter, and which, as we have seen, increase so much in frequency as the composition of the matter becomes more complex. When they come to the still more complex form of matter entering into the composition of protoplasm such transformations are no longer referred to. The matter itself is admitted to be of the most changeable kind, and the forms of living things are admitted to depend upon the intimate constitution of the units of living matter from which they arise (the "physiological units" of Spencer, or the "ids" of Weismann), and yet not one word is said as to the existence among organisms of transformations of form akin to those which we know as occurring in such simple substances as carbon, phosphorus, salts of mercury and other metals, as well as in the carbon compounds.

I, however, believe that analogous transformations of living matter are constantly occurring among lower forms of animal and vegetal life, and that these are represented by multitudinous heterogenetic transformations, some few of which have been studied in this work.

[1] See his "Principles of Biology," Revised Edition, 1898, vol. i., pp. 633-646. His own much simpler views concerning Heredity, based upon his conceptions concerning "organic polarity" and "physiological units," may be gathered from pp. 225, 315-319, 350-355.

These lower forms of life for the most part multiply, as I have said, by mere "discontinuous growth," and their forms are, like those of crystals, absolutely dependent upon their molecular constitution and the environing conditions in the midst of which they exist. Changes of this or that kind in their environing conditions, will, in many of them, lead to remarkable changes owing to alterations thereby induced in their molecular constitution—alterations which have as their result the establishment of new developmental tendencies. Moreover, I believe that many of the discrepant observations made by good observers as to the ordinary phases in the life-history of such pleomorphic species as Chlamydococcus, Pleurococcus, and many others, have been due to modifications occurring from time to time in their developmental phases, under the influence of special conditions, of one or another kind, though they may ultimately revert to the forms from which they started. The accounts that have been given by good observers show that a regular order is not observed, and strange phases are from time to time apt to be intercalated. Many of these lower forms of life are so much the creatures of circumstances—so transitory in their duration—that I long ago proposed to speak of them as "Ephemeromorphs," in contradistinction to the regularly recurring forms comprised under and understood by the term "species."

Weisman, on the other hand, altogether repudiates heterogenesis, or *per saltum* development. He even ventured to say that, "Cases of sudden transformation of the whole organism with subsequent inheritance are as yet quite unknown." Heterogenesis in lower organisms, such as I believe in, and such as I have made known in this work, he did not allude to, and probably altogether repudiated. Throughout his essay, in fact, he makes little reference to lower organisms with which, as I maintain, the phenomena are so common. He alludes principally to higher organisms; and what some would believe to be a striking transformation of this type, the now well-known metamorphosis of the Mexican Axolotl, he does his best to explain away. "It must most probably be regarded in a different light," is, however, all that he feels warranted in saying after the fullest discussion of the subject.[1]

From any point of view, however, this transformation, which has been so frequently observed, is one of the most remarkable on record. Weismann says (p. 577), "The structural differences between Axolotl and Amblystoma are considerably greater and of more importance than those between allied genera, or indeed than between the families of the Urodela." Yet changes so

[1] *Loc. cit.* pp. 555-633.

remarkable have often been observed to be brought about in periods varying from twelve to sixteen days.

There is also the fact recorded by Darwin[1] that on five separate occasions what Sclater has pronounced to be a distinct species of Peacock—the "black shouldered kind," or *Pavo nigripennis*—has appeared suddenly in a stock of common or pied peacocks, and in two of the cases (that is, in the flocks of Sir J. Trevelyan and Mr. Thornton) the black shouldered kind increased "to the extinction of the previously existing breed."

Then, again, as J. J. Murphy says[2] "The otter, or Ancon, sheep of North America was also the result of a sudden variation; and the differences in the form of its skeleton from that of the common sheep amounted to a specific if not a generic difference." It is true, as this writer says, these and other instances that could be mentioned have arisen under domestication.[3] But he pertinently asks why may not the same kind of thing be taking place in the wild state? "It may be true that we have no evidence of the origin of wild species in this way. But this is not a case in which negative evidence proves anything. We have never witnessed the origin of a wild species by any process whatever; and if a species were to come suddenly into being in the wild state, as the Ancon sheep did under domestication, how could we ascertain the fact? If the first of a newly-begotten species were found, the fact of its discovery would tell nothing about its origin. Naturalists would register it as a very rare species, having been only once met with, *but they would have no means of knowing whether it were the first or last of its race.*"

The instances just cited are cases in which new species have suddenly appeared without known cause—no one has been able to fix upon any external determining conditions. Other instances, however, may be cited where, as with the metamorphosis of the Axolotl, a more or less definite and known change of conditions has helped to bring about the transformations in question.

One such case, where a marked alteration in climate was operative, has been cited by Darwin.[4] Metzger obtained seeds of a tall kind of maize (*Zea altissima*) from the warm regions of America and cultivated them in Germany. During the first and second years the plants reared showed some differences from

[1] "Animals and Plants under Domestication," vol. i., p. 290.

[2] "Habit and Intelligence," 1869, vol. i., p. 343.

[3] "It is certain that the Ancon and Mauchamp breeds of sheep, and almost certain that the Niata cattle, turnspit and pug-dogs, jumper and frizzled fowls, short faced tumbler pigeons, hook-billed ducks, &c., and with plants a multitude of varieties, suddenly appeared in nearly the same state as we now see them." (Darwin, *loc. cit.*, vol. ii., p. 414.)

[4] "Animals and Plants under Domestication," vol. i., p. 322.

the parent stock, and, "In the third generation nearly all resemblance to the original and very distinct American parent-form was lost. In the sixth generation this maize perfectly resembled a European variety. . . . When Metzger published his book, this variety was still cultivated near Heidelberg, and could be distinguished from the common kind only by a somewhat more vigorous growth." Darwin speaks of this as the most remarkable instance known to him "of the direct and prompt action of climate upon a plant."

Another case even still more remarkable and convincing has been cited by Wallace[1] from Semper's work on "The Natural Conditions of Existence as they Affect Animal Life," (1883). He says: "Perhaps the most remarkable case he has brought forward is that of the transformation of species of Crustaceans by a change in the saltness of the water. *Artemia salina* lives in brackish water, while *A. Milhausenii* inhabits water which is much salter. They differ greatly in the form of the tail-lobes, and in the presence or absence of spines upon the tail, and had always been considered perfectly distinct species. Yet either was transformed into the other in a few generations, during which the saltness of the water was gradually altered. Yet more, *A. salina* was gradually accustomed to fresher water, and in the course of a few generations, when the water had become perfectly fresh, the species was changed into *Branchipus stagnalis*, which had always been considered to belong to a different genus on account of the differences in the form of the antennæ and of the posterior segments of the body. This certainly appears to be a proof of change of conditions producing a change of form independently of selection, and of that change of form, while remaining under the same conditions, being inherited."

It is certainly strange in the face of these facts, all of which, with the possible exception of the last, would have been known to Prof. Weismann, that he should have said, "Cases of sudden transformation of the whole organism with subsequent inheritance are as yet quite unknown." Whatever he may mean by "sudden," most persons would consider that the cases above cited have belonged to this category.

But other new instances of a remarkable kind have been made known during the last two years by Prof. de Vries, of Amsterdam,[2] whose experiments, extending over several years, with one of the Evening Primroses, *Œnothera Lamarckiana*, have revealed the sudden appearance among its progeny of no less than seven distinct species. The representatives of these

[1] "Darwinism," 1890, p. 427.

[2] "Die Mutationstheorie," Leipzig, 1901.

new species severally produced descendants, the majority of which unmistakably adhered to their respective types. Variations, however, were encountered in this respect. Some of the new species, like *Œ. gigas*, were found to be very stable forms. On the other hand, *Œ. scintillans* was extremely unstable, *i.e.*, possessed the property of mutation to a high degree, a large proportion of its descendants belonging to other species, especially *Œ. oblonga*, and *Œ. Lamarckiana*.

The first of these cases of transformation to which I have referred, that of the Mexican Axolotl, is altogether exceptional and peculiar, since the change begins when the organism is already approaching its full growth and development. And whether or not it is to be regarded as an instance of "reversion," the phenomena are almost equally remarkable. A change, initiated in one part of the organism with the loss of its gills, gradually spreads to the organism as a whole by a process which Darwin calls "correlation," and Herbert Spencer speaks of as "organic polarity"—by the same kind of process, in fact, though infinitely more complex, as that which may lead a crystal under change of conditions to assume an entirely different form and appearance (see p. 327).

In the other cases we can only suppose that some isomeric change has taken place in the germ, that is, in its constituent "physiological units," as a consequence of which in the course of development it has gradually unfolded into a form more or less new.

It is only to be expected that among higher organisms, whether plants or animals, such changes should be comparatively rare and comparatively slight. As Herbert Spencer says: "That organic types should be comparatively stable might be anticipated on the hypothesis of Evolution. If we assume, as we must according to this hypothesis, that the structure of any organism is a product of the almost infinite series of actions and reactions to which all ancestral organisms have been exposed, we shall see that any unusual actions and reactions brought to bear on an individual can have but an infinitesimal effect in permanently changing the structure of the organism as a whole. The new set of forces, compounded with all the antecedent sets of forces, can but inappreciably modify that moving equilibrium of functions which all these antecedent sets of forces have established."

But where there has been an absence "of the almost infinite series of actions and reactions," where the ancestral series has been one in which mere "discontinuous growth" has been the rule, the conservative influence of heredity would be comparatively absent, and plastic living matter in its lower forms, such as we find it among the great assemblage of "Ephemeromorphs,"

would be much more open to have variations impressed upon it, and to exhibit heterogenetic transformations, such as we have been concerned with in this volume—transformations that are, as I believe, but a continuation into a higher platform of those allotropic and isomeric metamorphoses with which we are so familiar in not-living matter.

Let us turn now to enquire *why* it is that Weismann is led to regard Heterogenesis or *per saltum* development as an untenable doctrine.

What I have already pointed out must, in the first place, be borne in mind, namely, that he does not deal with Heterogenesis as it is alleged to occur among lower forms of life. In regard to this side of the question he is silent. He sets himself especially to rebut the views of Von Hartmann and others as to the existence of a special "phyletic vital force," or "internal principle of development," but in so doing he proclaims his objections to heterogenetic and *per saltum* transformations generally.

I am not a believer in the existence of any alleged "phyletic vital force"; but as a believer in heterogenetic transformations of organisms I am concerned to show that the principal reasons relied upon by Weismann against the occurrence of such transformations generally (especially in their application to lower organisms and apart altogether from Von Hartmann's doctrines) are open to grave objections. That his criticism is intended to have a general application is expressly stated, seeing that he says (p. 698): "It nevertheless appears to me not to be superfluous in such a deeply important question to show that a *per saltum* development, and especially the so-called heterogeneous generation, is inconceivable, not only on the ground of the arguments formerly employed against the phyletic force in general, but quite independently of these."

We may admit with Prof. Weismann that "alternation of generation" is not a case in point, on account of the cyclical nature of the changes, but when he says, "All other facts which have hitherto been referred to 'heterogeneous generation' are still less explicable as such, inasmuch as they always relate to change in single parts of an organism, such as the sudden change of fruit or flower in cultivated plants," it must be clear from what has gone before that such a statement is actually erroneous.

Then, again, when he says "if we nowhere see sudden variations of large amount perpetuated by heredity, &c." (p. 701) he is clearly making a statement which no longer holds good; as also when he says *per saltum* variation is "unsupported by a single observation," and that the supposed 'heterogeneous generation' is always illustrated by the example of alternation of generation." As against Von Hartmann this latter statement

may be true; but as generally applicable it is as far from being exact as the others which have preceded it.

Then, at last, we come to an argument in place of statements which can only be described as erroneous. Taking a case where "it is imagined that one animal form suddenly gives rise to another widely deviating form by purely internal causes," Prof. Weismann says (p. 703): "Now on this theory it would be an unavoidable postulate that by such a process of *per saltum* development there arises not merely a new type of some species, but at the same time individuals capable of living and of persisting under, and fitted to, given conditions of life. But every naturalist who has attempted to completely explain the relation between structure and mode of life knows that even the small differences which separate one species from another always comprise a number of minute structural deviations which are related to well-defined conditions of life—he knows that in every species of animal the whole structure is adapted in the most exact manner in *every detail* to special conditions of life."

To these statements from a general point of view the answer is threefold:—

(1) Changes never can be brought about by "purely internal causes"; in all cases, with organisms as with crystals, the form assumed must be the result of internal changes acting under the influence of the sum-total of external conditions.

(2) What is said as to the need of special adaptation to conditions does not apply, except with great limitations, to the lowest forms of animal and vegetal life which are known to have the most widespread geographical distribution and therefore to be capable of existing under very varied conditions. Even in an organism so high as a Nematoid it is notorious that representatives are met with leading a parasitic life which are almost indistinguishable from many of the free, non-parasitic forms.

(3) Then again, the facts which I have cited concerning the "black shouldered" Peacock, the Ancon sheep, the tall American maize, the crustaceans of the genus Artemia, and the various species of Œnothera, are so many examples of transformations more or less rapidly brought about, not of course due to internal changes alone but to the conjoint influence of internal tendencies and external conditions operating at the time—the latter being an aspect of the problem which Weismann ignores, or he would never have made such a statement as this: "When by germinal metamorphosis a new form has arisen, this, from the first moment of its existence must be adapted to the new conditions of life or it must perish." The cases above referred to have doubtless been cases of germinal metamorphosis, and they have been so far adapted to their conditions of life, that instead of perishing they have increased, multiplied and perpetuated their kind.

Moreover, as J. J. Murphy says, who can tell how often such processes occur among animals and plants in the wild state, when the appearance of new species in this manner must always be incapable of verification?

Again, Prof. Weismann writes "the abrupt transformation of species implies sudden change in the conditions of life," to which one can only reply that it has not always been so; as evidence of which there has been on different occasions amidst the old conditions the appearance of the "black shouldered" Peacock, and the appearance of new species of Œnothera. And when he goes on to say, "if such abrupt transformation takes place it must produce the new form instantly equipped for the struggle for existence, and adapted in all its organs and systems of organs to the special conditions of its new life," I rejoin that this does not in the least apply to the appearance, by comparatively abrupt transformations, of multitudes of the lower forms of life such as Amœbæ, flagellate Monads, Moulds, Peranemata, Ciliated Infusoria, and many other of the lower types which are by no means so dependent upon specialised external conditions, and amidst which the part played by Natural Selection is reduced to its lowest terms.

These mere inaccurate statements and one-sided arguments, as I am compelled to call them, are all that Prof. Weismann in the course of eleven pages is able to say against the occurrence of a *per saltum* development, as one of the modes in which Evolution is carried on. His statements and arguments must, I think, be absolutely unconvincing to any open-minded critic.

So far from there being any *à priori* objections to *per saltum* development or Heterogenesis, which, as I claim to have shown, occurs so frequently, the fact of its occurrence ought to be considered thoroughly harmonious with all that we know concerning simpler forms of matter, and with the constitution of living matter itself.

As Herbert Spencer says,[1] it is a cardinal fact "that proteids admit of multitudinous transformations; and it seems not improbable that in protoplasm various isomeric proteids are mingled. If so we must conclude that protoplasm admits of almost infinite variations in nature." But the "ids" of Weismann as well as the "physiological units" of Herbert Spencer are essentially protoplasmic in nature and therefore of great molecular complexity. These are the ultimate units, the "unknown somethings which have the power of organising themselves into a structure of this or that species."[2] I merely contend, therefore, that these ultimate units, looking to their

[1] "Principles of Biology," vol. i., 1898, p. 67.

[2] *Loc. cit.*, p. 370.

nature and origin, must be supposed to be capable of undergoing isomeric changes within certain limits, and that, such changes having been brought about, these altered units would tend to unfold into new forms—more or less different from the parent forms, and corresponding with this or that kind of heterogenetic transformation. The number of such transformations now known is considerable among lower organisms, though comparatively rare among higher plants and animals. But the range of variation is also great among lower forms of life and comparatively slight in the case of higher types.

The rule hitherto has been that each announcement of any such transformation has been received with the greatest incredulity, and efforts have always been made either to discountenance its reality or to minimise its importance, owing to the fact that the ultimate developmental units of living things, though composed of a matter which "admits of almost infinite variations in nature," have not in practice been credited with any such properties. These developmental units have been deemed to be fixed and comparatively unalterable under the controlling influence of heredity, an influence which undoubtedly tends to increase as higher grades of organisation are attained—but which seems to be comparatively weak among the lowest types of living things.

If the views that I have been advocating seem difficult of acceptance to many let them keep steadily in mind all that has been adduced, as matter of common knowledge, concerning the isomeric states of various saline substances, their transformations in crystalline form, in colour, and in other characters—under variations in external conditions; as well as the total transformations of such elementary substances as carbon, phosphorus and sulphur owing to alterations in the exact collocation of the atoms in their component molecules.

The alleged facts concerning heterogenesis may be said to be inconceivable. Granted : but that is no reason for doubting their validity. How far are processes of allotropism and isomerism conceivable in any true sense of the word. Our notions of conceivability in regard to physical phenomena have received many severe shocks even during the last twelve months—by revelations concerning wireless telegraphy, the "mystery of radium," and the constitution of matter itself—that is, of the "simple atoms" of which elementary substances are composed. Thus, Sir Oliver Lodge tells us that we are now arriving at an electrical theory of matter; [1] that in an atom of hydrogen there are nearly 1,000 electrons, and in the mercury atom

[1] See *Nature*, March 12, 1903.

100,000 electrons. But the electrons are so minute that even with these vast numbers in a single atom, they do not "fill all the space, and if the distance between them were calculated, they seemed to be about as far apart in proportion to their size as the planets in the solar system." While Professor Rutherford[1] has shown that the behaviour of radium, as well as of thorium and uranium, is only to be explained on the idea that they are elements in the process of slow "spontaneous transformation." Radium emits three kinds of rays, and the most important of them are what have been named *alpha* rays, "which although less striking than either of the other two kinds to the casual observer, have proved to be the most wonderful of all. For they are different from anything else that has ever been dealt with in science before. Rutherford early this year showed that they are not rays in the ordinary sense at all, but matter in the form of single atoms, comparable with the hydrogen atom in size, radially projected into space with the stupendous velocity of 30,000 miles a second." These particles are said to be a thousand times more massive than the electron, and to move a hundred times faster than the fastest flying star. And during this process of molecular disintegration radium, like its allies, "*may be looked upon as continuously giving rise to new elements by a process of material evolution.*" One of these new elements has now been definitely ascertained by Sir William Ramsay to be the well-known gas helium. Doubtless other of the products will soon be discovered, and it will be generally recognised that even the "spontaneous generation"—hitherto unknown—of so-called chemical elements is, under suitable conditions, ever taking place around us.

After such revelations, who would dogmatise and attempt to set bounds to the potencies of matter, living or not living? Let us think of what is known as to the great complexity of the molecules of proteid substances, and the further extreme complexity of the units of living matter which they help to form. Let us then attempt to add to this conception a further conception based upon these modern revelations as to the constitution of even the simplest atom entering into the composition of protoplasm, and, even if willing to accept what is attested by observation and experiment as to the heterogenetic transformations of living matter, we may be perfectly free to admit our utter inability to explain the mutations in question.[2]

[1] See an article on "The Disintegration Theory of Radio-activity," *The Times Literary Supplement*, June 26, 1903.

[2] Our difficulties as biologists are far surpassed by those at present confronting physicists and chemists. C. V. Boys in his recent Presidential Address to *Section A* of the British Association said (*Nature*, September 10, 1903, p. 448):—"The discovery of Professor and Madame Curie of what

XXVI.—CONCLUSION.

In the foregoing pages I have shown that Bacteria of different kinds, Moulds, and other varieties of Fungi may, and undoubtedly do, arise *de novo* by heterogenesis; that various simple Algæ, Diatoms, and Phytozoa may take their origin from alien sources; and that a similar heterogenetic origin is most frequently met with for Amœbæ, Actinophrys, Flagellate Monads, Peranemata, and even Ciliated Infusoria.

The heterogenetic origin of these organisms takes place after one or other of the four following methods:—

(1) They may arise, as with Bacteria and some Amœbæ, by the gradual growth of previously invisible microscopic particles revealing themselves in the substance of their matrix.

(2) They may arise from the fusion and subsequent individualisation of groups of Bacteria, as in the origin of Flagellate Monads, Amœbæ, Fungus germs, and Ciliated Infusoria from transformed *Zooglœa* aggregates in the pellicle on a hay or other organic infusion.

(3) They may take origin, as with other Amœbæ, Flagellate Monads, Actinophrys, Peranemata, Fungus germs, and Ciliated Infusoria, by a simultaneous segmentation of the entire substance of some matrix into a number of more or less equal parts, each of which develops into similar representatives of one or other of these forms of life.

(4) Or, the whole of a given matrix may be transformed into a new form of life, as when Chlorophyll Corpuscles are converted into Amœbæ, Actinophrys, or some of the simpler Algæ; when the cells of a parasitic Alga are converted into Diatoms or Euglenæ; or when the entire egg of a Rotifer or of a Tardigrade becomes transformed into one of the larger forms of Ciliated Infusoria.[1]

I have shown, moreover, how convertible many of these alien derivatives are—how in the pellicle on a hay infusion masses of *Zooglœa* are formed which segment now into Fungus germs, now

seems to be the everlasting production of heat in easily measurable quantity by a minute amount of a radium compound is so amazing that, even now that many of us have had the opportunity of seeing with our own eyes the heated thermometer, we hardly are able to believe what we see. This, which can barely be distinguished from perpetual motion, which is an axiom of science to call impossible, has left every chemist and physicist in a state of bewilderment. . . . Theories are being invented to account for the marvellous results of observation; but the theories themselves would a few years ago have seemed more wonderful and incredible than the facts, as we believe them to be, do to-day."

[1] The evidence in support of these statements, and others in subsequent paragraphs, may be easily found by consulting the Index.

into Flagellate Monads, and now into Amœbæ; and similarly, that the substance of a Rotifer's egg may under different conditions segment into Flagellate Monads, Amœbæ, Peranemata, or even into Ciliated Infusoria; that on different occasions one or other of the same kinds of organisms may take origin from the substance of large encysted Amœbæ; while an encysted Ciliate may also itself break up into segments which develop sometimes into Flagellate Monads, and at others into Amœbæ, Peranemata or Fungus germs.

I have further shown that Confervoid cells, "resting spores" of Spirogyra, and "resting spores" of Vaucheria may also yield Amœbæ, Actinophrys, or Flagellate Monads; and I have demonstrated how abundantly similar organisms are born within the closed cells of Nitella, and Spirogyra, and the filaments of Vaucheria. Again, we have found all these organisms—Fungi, Monads, Amœbæ, Actinophrys and Ciliated Infusoria proceeding from the substance of Euglenæ; while at other times various Algæ are seen to be derived either from their transformation as a whole or from that of their Chlorophyll Corpuscles.

Finally I have shown there is no escape from the conclusion that different kinds of Bacteria may take their origin within the tissues and cells of animal organisms; and that Bacteria and their allies, as well as the simpler forms of Fungi, may have a similar *de novo* origin within the closed cells of many vegetables and fruits.

However astounding these statements may seem to those who have not worked long and earnestly at such subjects for themselves, I venture to submit that the facts adduced in this volume, backed by the appearances preserved in the photomicrographs, should go far to convince those who will give the subject a fair and impartial consideration. The appearances thus recorded and preserved can neither be explained by the facile solution that they are "results of infection," nor can it be said that the new forms of life which seem to arise by heterogenesis are in reality normal and habitual phases in the life-history of the organisms from which they proceed. These other possible interpretations have always been carefully kept in view, and the evidence for and against rival interpretations has in each case been duly weighed.

Still, as has been said on other occasions, it will doubtless be said again that the facts I have brought forward are mere figments of my imagination, seeing that others observe no such phenomena, and that my experience is altogether exceptional. As to this I have three replies to make.

In the first place, my experience is not so exceptional. There are numberless instances recorded in botanical and zoological

memoirs (to some of which I have referred) in which transformations such as I have described have been recorded. But they have almost invariably been assumed (without any attempt at proof) either to be instances of infection by parasites, or of the production of hitherto unknown reproductive units of the organisms in which they have been found.

Secondly, the reason why I have met with such numerous instances of what have hitherto been described in one or other of these ways, is that I have most diligently looked for them during many years, and have moreover sought to produce them experimentally by keeping various organisms exposed for different periods to one or other unnatural set of conditions.

My third reply to these critics is, that in order to meet their facile objection that I am a biassed observer I have purposely had recourse to photomicrography, and can thus boldly ask whose interpretation of the appearances which the figures present is most in accordance with actual facts and existing knowledge?

Then, again, some of my critics have refused to give any adequate consideration to the work because it has not been entirely done under certain impossible conditions which they would prescribe. They are disposed to repudiate my observations on Heterogenesis, either (*a*) because I have not generally adopted such methods as are had recourse to by bacteriologists in their investigations, or have not uniformly isolated the organisms whose changes have been under examination; or (*b*) because the observations of the various stages of change have not been made continuously upon the same individual organisms, but have been pieced together by the examination of different individuals (generally lying side by side) in successive stages of transformation.

(*a*) But in regard to this first objection, it will be seen that it is inapplicable to all the observations recorded in Section xxiii. in proof of the heterogenetic origin of Bacteria and their allies, seeing that these observations have been conducted with all necessary precautions against the possibility of infection. While in regard to many other observations that have been made—touching the heterogenetic origin of Amœbæ, Monads, Peranemata and Ciliated Infusoria—it must be seen that these, if made at all, have to be made on dying organisms either in their own or in experimentally varied environments; that in either case aseptic conditions or absolute isolation of the organisms under observation is always an impossibility; and that any attempts in this direction would inevitably stop the changes hitherto in progress.

The attempt, indeed, to enforce such requirements, and the complete disregard of the actual nature of the facts alleged, can only be looked upon as the strongest evidence of bias on the part of those who make it. They are shown (by the aid of photo-

graphs) encysted Ciliates dividing simultaneously, and without remainder, into spherical masses which develop into Amœbæ, Monads or Peranemata; they are shown the substance of great Amœbæ segmenting and yielding Ciliated Infusoria; and, again, they are shown similar segmentations occurring in the substance of Rotifers' eggs, with the production therefrom of one or other of the same kinds of organisms, and even the conversion of the entire substance of a Rotifer's egg into a great Ciliated Infusorium. Yet little or no notice is taken of such observations. The learned Societies will not consider them, or allow them to be discussed. I maintain, however, that no explanation can be given of such phenomena, consistent with reason and actual knowledge, by those who are so supercilious in regard to the plain interpretation offered by me.

(*b*) In regard to the objection that there has not been a continuous watching of the alleged heterogenetic changes from start to finish on the same individual organism, I can only say that compliance with such demands would not only be fruitless but would go far to render for ever impossible any knowledge of heterogenesis. I submit that such evidence as has been brought forward in this volume is the only kind of evidence that can be adduced in proof of heterogenesis. Moreover, the observation of different stages of change in different individuals is, after all, the mode by which, as is well known, multitudes of embryological investigations have to be conducted. The methods employed by those who would gain a knowledge of heterogenesis, cannot, from the very nature of the subject, be strict laboratory methods; but they are none the less methods similar to those by which much other scientific knowledge has gradually been built up. Those who work at this subject have to adopt methods which, though carrying with them a greater element of certainty and a larger amount of actual observation of the processes in question, are fairly comparable with those employed by geologists —we each of us strive to put the best and most reasonable interpretation upon the facts that come under our observation, as much as possible irrespective of preconceptions and *à priori* views.[1]

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[1] Those who are sceptical concerning Heterogenesis and have any desire to be convinced may easily, by following my instructions, study the changes occurring in *Zooglœa* masses in the pellicle on a hay infusion and the appearance of Ciliated Infusoria therein; or they may study the origin of Diatoms from cells of Chlorochytrium in dead Duckweed, the origin of pigment Amœbæ in Vaucheria filaments or "resting spores," or some of the remarkable changes which I have described as occurring within the cells of Nitella. All these are transformations that can be induced or found almost at will. Others, as I have shown, are more fitful in their occurrence, or can only be met with under conditions not always easy to realise, as with the transformation of Hydatina eggs into Ciliated Infusoria, and many other of the changes which I have recorded.

The close inter-relations and interchangeability that I have shown to exist between the varied lower types of life with which we have been concerned forces upon us the belief, that the actual forms assumed are as much the immediate results of their molecular composition and their environing forces as are the various forms assumed by crystals.[1] We are driven to the belief, in fact, that "organic polarity" is the dominating influence in the production of this or that form among these lower organisms forming part of the great assemblage for which I long ago proposed the name "Ephemeromorphs";[2] that heredity in any other sense is not operative among them; and that this whole world of lowest vegetal and animal forms of life must be wholly removed from the influence of "natural selection."

I am well aware that these are very unorthodox views—that Darwin, Weismann, Poulton and others actually believe that the the rate of change is lower among lowest than among higher organisms. This most surprising view is based in the main upon the fact dwelt upon by Huxley and others as to the "persistence of types" through geologic ages—a fact which, with the proof of the occurrence of heterogenesis, is capable of receiving a totally different interpretation, and one that is in no way inconsistent with the recognition of all that has been shown by multitudes of workers as to the extreme variability of these Ephemeromorphs, and the polymorphism of such organisms as Protococcus, Hæmatococcus, Chlamydomonas, Sphæroplia and others. The changes among these latter organisms are indeed so fitful, and vary so much in their order at different times and seasons (as witness different observers) that their varying successive changes could not be considered to be results of heredity. Like actual heterogenetic transformations, these irregular variations must be considered to depend upon slight isomeric changes in the molecular constitution of what Herbert Spencer terms their "physiological units," acting in conjunction with organic polarity as a formative principle.[3]

The extreme variability of the organisms referred to above is well illustrated by the researches of F. Cohn on *Protococcus pluvialis;* and his observations may be cited as an example of many others.[4] He has shown, as derivatives of this apparently

[1] See *Appendix*, p. ii.

[2] "The Beginnings of Life," 1872, vol. ii., pp. 559 and 600.

[3] May we not also account in the same way for those "individual variations" that are continually occurring among all higher forms of life, and which as Weismann says ("*Germ Plasm,*" p. 410), "after the precedent of Darwin and Wallace we regard as the foundation of all processes of natural selection"—though he accounts for these variations quite differently.

[4] See Transln. of his memoir, with Plate, in "Botanical and Physiological Memoirs," Ray Soc. 1853. The reader may also consult Cook's "British Fresh Water Algæ," for details as to the great variability of the other forms mentioned above.

simple Alga, the most widely differing forms of Phytozoa, which were actually seen by him to be derived from one another by direct processes of transformation. In summing up the results of his investigation he says:[1] "Thus we see that a single species, owing to its numerous modes of propagation, can pass through a number of very various forms of development, which have been either erroneously arranged as distinct genera, or at least as remaining stationary in those genera, although, in fact only transitionary stages." Then, after a brief enumeration of the principal changes he has witnessed among the derivatives of this simple Alga, he adds the following important statement: "A critical and comparative consideration of the foregoing facts would therefore appear to render untenable almost all the principles which modern systematists have hitherto adopted as the basis for the construction of their Natural Kingdoms, Families, Genera, and Species."

It seems clear, in fact, that so long as organisms multiply by mere "discontinuous growth" (fission or gemmation) there is absolutely no reason to appeal to heredity except in the form of "organic polarity." This would be all-sufficient for the preservation of their likeness under similar conditions, to account for their changes under dissimilar conditions, and for their reversion (as with crystals and double salts)[2] to old forms on renewal of old conditions—a class of facts with which the researches of many bacteriologists have made us perfectly familiar.

It is, therefore, not till we get out of the great Ephemeromorphic plexus, and come to organic forms which habitually multiply by sperm cells and germ cells (or occasionally by the latter alone) that heredity, as postulated by Darwin and Weismann, comes into play: and it is consequently not till we reach such forms that it is possible for "natural selection" to be influential as a cause of evolution. I make bold to say this notwithstanding the opposite views held by Darwin himself, Weismann and others who, as is well known, maintain that the influence of natural selection as a producer of fitness between organisms and their circumstances is one which is applicable to all forms of life.

The evidence for the conjoint association of organic polarity and heterogenesis is far more certain, and indeed belongs to a wholly different order of certitude from that upon which the co-operative alliance between heredity and natural selection depends. On the one hand we have the facts directly observed and recorded in this volume; while on the other Weismann himself says,[3] "It is true that we have never directly observed

[1] *Loc. cit.*, p. 559. [2] See p. 328.

[3] "Studies in the Theory of Descent," Trans., p. 643.

the process of natural selection" and he then goes on to illustrate the kind of evidence that exists for it, and its cogency, by adding, "neither has anybody directly observed the mode in which the heat of the animal body is generated by the processes of combustion going on in the blood and in the tissues, nevertheless this is believed as a certainty." We see, therefore, that for the establishment of both these doctrines although the amount of direct observation has been altogether inadequate to bring about conviction, the lacunæ, as in scientific investigations generally, have been filled by the exercise of reason. Yet the tendency with my critics has been to demand nothing but observation, "continuous and unbroken" (p. 130) and to allow no place whatever for reason, even to supply the smallest links in the chain of evidence. That this is no exaggerated statement anyone may see for himself who will carefully peruse Appendix II., and contrast the evidence which I adduced as to the reality of the transformation of Hydatina eggs into great Otostomata, and the incredulity with which this evidence has been received not only in this country but abroad.

* * * *

While my views are in opposition to doctrines that have long held sway, I have at least the consolation of feeling that they are consistent with my position as a believer in the evolution hypothesis. As much cannot be said for the consistency of the views of many of my critics. While disposed to discredit my observations, they never deign to give any adequate explanation in harmony with their own views concerning evolution, as to why such multitudes of the lower forms of life exist at the present day; why some portions of a living matter evolved, as they believe, in the past by natural processes, should have gone through all the marvellous phases of development represented by past and present forms of life, both animal and vegetal, while other portions of this confessedly highly mutable living matter should have remained through untold ages in one or other of its lowest developmental phases. My answer to this great problem, I would submit, is both founded upon fact and consistent with reason: *lowest organisms exist at the present day because they are ever seething up anew by processes of Heterogenesis;* moreover, it seems probable that they have been similarly appearing in all ages since living matter first became common on the surface of this earth.

These beliefs carry with them considerations of the utmost importance in regard to the palæontological history of our globe and the present distribution of life upon its surface.[1] They

[1] See *Appendix* I., pp. v-vii.

throw light especially upon the cause of the presence of those "persistent types" of life through long geologic ages to which I have already adverted. This persistence is met with in some organisms very much higher than any with which we have been concerned in this volume. Thus, to cite one group of examples only from the numerous cases referred to by Huxley,[1] we find that authority writing as follows: "Turning to the *Mollusca*, the genera Crania, Discnia, and Lingula have persisted from the Silurian epoch to the present day with so little change that competent malacologists are sometimes puzzled to distinguish the ancient from the modern species."

Although it is a matter of extreme interest that organisms so high as these Molluscs should present themselves in almost identical forms through this vast series of ages, what is of more immediate interest to us is the fact that two families of such organisms as are comprised within the great Ephemeromorphic plexus have their remains, in the form of their calcareous or siliceous envelopes, abundantly preserved in the crust of the earth. I refer to Foraminifera and to Diatoms—representatives respectively of Protozoa and Protophyta.

Concerning the former organisms Dr. Carpenter wrote,[2] "there is no evidence of any fundamental modification or advance in the Foraminiferous type from the palæozoic period to the present day." Similar types and similar varieties from those types are, he said, to be met with in geological formations existing as far back as the triassic rocks. And he adds, "The only natural classification of the vast aggregate of diversified forms which this group contains, will be one which ranges them according to their direction and degree of divergence from a small number of principal family types." In reference to the same subject Pritchard says[3]: "It may be generally stated that the relative number of identical fossil and recent species is much greater in this family of Foraminifera than in any other known; and specific forms have continued from the Mesozoic era until the present day, so connecting, as by an unbroken chain, the fauna of our own time and that of almost countless ages past."

Turning now to the Diatomaceæ, we find similar evidence as to their cosmopolitan distribution and as to the essential similarity of the fossil species with those existing at the present day. The extreme variability of these organisms is also well known. Sir Joseph Hooker in his report on the Diatomaceous vegetation of the Antarctic sea wrote as follows[4]: "The genera

[1] *Proceed. of Royal Institution*, vol. iii., p. 151.

[2] "Introduction to the Study of the Foraminifera," 1862, p. xi.

[3] "History of Infusoria," Fourth Edition, p. 232.

[4] *British Association Report*, 1847.

and species of Diatomaceæ collected within the Antarctic sea are not at all peculiar to those latitudes; on the contrary, some occur in every country between Spitzbergen and Victoria Land. Others, and even some of these, have been recognised by Ehrenberg as occurring fossil in both Americas, in the south of Europe and north of Africa, in Tripoli stone and in volcanic ashes both from active and extinct volcanoes, whilst others again exist in the atmosphere overhanging the tropical Atlantic." And, in reference more especially to the agreement of recent and fossil forms, Dr. Gregory[1] wrote: "I have no hesitation in saying that I believe all the forms in the Ægina clay-marl, which is the oldest Diatomaceous deposit yet described, will be found living on our coast[2]. . . . It may also be observed that, of all the forms figured by Ehrenberg from more recent strata. . . . the great majority are perfectly identical with existing Diatoms." He subsequently adds: "Taking these facts into consideration, I am led to believe that we have no evidence that any species of Diatom has become extinct, as so many species, and even genera and tribes, of more highly organised beings have done."

The wide geographical distribution of these as well as of other of the lowest forms of life over the surface of the earth at the present day, and the fact that they show themselves in the most varied regions with identical anatomical characters, seems to indicate pretty conclusively that the intrinsic properties and varieties of the matter of which they are composed (*i.e.*, of their "physiological units"), have more to do with their forms and structures than any differences in the conditions under which they have been born and have lived. The same conclusion may be drawn from the fact of the similarity of the forms of Diatoms and of Foraminifera that lived upon the surface of the earth or in its oceans in remote ages, with those existing at the present day.

Are we to assume, however, that these and other low organisms with which we have been concerned in this volume have had an unbroken lineal descent through the millions and millions of years since they first came into existence? This is the commonly received notion, upon which, moreover, has been based the fallacious conclusion (otherwise so much at variance with known facts) as to the slow rate of change among these lowest organisms —a view leading Poulton to believe that such enormous periods of time would be needed to account for the evolution of all the forms of life upon the globe.[3] But if this lineal descent had been the rule in past ages we might at least expect at the present day

[1] *Proc. Roy. Soc.*, Edinburgh, 1856-7, p. 442.

[2] The stratum at Ægina is said to belong either to the chalk formation, or to the oldest tertiary or Eocene beds.

[3] See *Appendix* I., p. ix.

to find such evidence of this continuity, as would be represented by fixity of habitat. Fixity of habitat is, however, notoriously non-existent among these lower forms of life. Speaking of Rotifers, Pritchard says:[1] "One remarkable circumstance must be borne in mind by the animalcule hunter. If he happens to remember a pond where some rare species abounded last year, let him not again turn thither in search of it, as the chances will not be in his favour. These creatures rarely exist in the same water during two successive years. The reasons for this are not easily ascertainable. The remark is equally applicable to Volvox and the Desmidiæ. The search will be most productive if prosecuted on new ground." Statements of this kind have also been made by others and are fully borne out by my own experience.

Ralfs, for instance, in reference to some Desmids, says:[2] "The *Staurocarpus cœruleus* is not uncommon near Penzance, is generally in large quantity where it occurs, and from its peculiar colour cannot escape detection; on these accounts I made it a principal subject of my observation. Although I have yearly gathered it in several pools, and the sporangia are always abundantly produced, I have particularly noticed during five or six years' observation that it has never in a single instance reappeared in the same pool. At Dolgelly, where also in some years it is common, I met with the same result, with a single exception, when I gathered it in one pool for two successive years. I have noticed the same fact with regard to *Zyngema curvatum*, and I believe it holds good in regard to most if not all the other Conjugatæ."

In the face then of this absence of continuity of habitat in the present, and of the frequent extreme variability of the lower forms of life—some indications of which have been given in the foregoing quotations from F. Cohn, Carpenter and others, together with what has been said as to the variability of Algæ generally (pp. 186-188)—how is it possible to accept the notion of direct lineal descent without change, as the explanation of "persistence of types"?

As I long ago pointed out[3] persistence of low types of life is much more explicable on "the assumption of successive evolutions of more or less similar forms from similar starting points under the influence of like conditions, than on the assumption that such changeable forms should have produced their like through such vast and unrealisable epochs of time." Persistence of types among lower forms of life is, in fact, to be expected in accordance with my views, seeing that the living things that

[1] *Loc. cit.*, p. 655.

[2] "The British Desmidiæ," 1848, p. 14.

[3] "The Beginnings of Life," vol. ii., p. 616.

have been constantly arising by Archebiosis and Heterogenesis have been the immediate products of ever acting material properties or natural laws—the same in all times, however much or little the environing conditions may have varied from age to age. On the other hand, to suppose that the representatives of the persistent types of life referred to have been all direct lineal descendants of some original forms which, once for all, in a remote past assumed their respective characters and have ever since continued to perpetuate their kind without change seems absolutely inexplicable from the point of view of the evolution philosophy.

LIST OF ILLUSTRATIONS.

A mere list of Illustrations is here given together with the Enlargements in Diameters of the several objects represented.

For the description of the figures the reader is referred to the text itself, at the pages indicated here, and on the plates after the numbers of the respective figures.[1]

PLATE XVII.

[1] The essential parts of the different Photographs have been cut out and placed side by side in groups. The Plates in this Fourth Part are, therefore, really made up of 153 Figures taken from separate Photomicrographs.

PLATE XVII.

PLATE XVIII.

SOME OF THE PHENOMENA THAT WILL BE RENDERED MORE EXPLICABLE BY THE ESTABLISHMENT OF THE DOCTRINE OF HETEROGENESIS.[1]

Explanation of the existence of Lowest Organisms at the present day.

The vast importance of this subject cannot fail to be recognised since, if my observations and views concerning the prevalence of Heterogenesis are correct, we may find at once a ready explanation of the existence at this period of the earth's history of the teeming multitudes of the lower forms of life whose presence must otherwise be logically considered such a stumbling block in the way of the acceptance of the doctrine of evolution.[2] To ask a person to believe that all the forms of life on this planet have during countless ages been evolved from the primordial living matter which first appeared thereon, and at the same time to expect him to believe that the causes of this evolution have through all these ages been inoperative upon myriads of the most primitive and modifiable forms of life, has always seemed to me, in spite of the attempts at explanation that have been made, most strange and unreasonable.

The questions which I put nearly thirty years ago have still to be repeated.[3] "Do not the very simplest forms of life abound at the present day? And would the Evolutionist really have us believe that such forms are direct continuations of an equally structureless matter which has existed for millions and millions of years without having undergone any differentiation? Would he have us believe that the simplest and most structureless Amœba of the present day can boast of a line of ancestors stretching back to such far-remote periods that in comparison with them the primeval men were but as things of yesterday? The notion surely is preposterously absurd; or, if true, the fact would be sufficient to overthrow the very first principles of their own Evolution philosophy."

On the other hand, if it be granted that, by processes of Heterogenesis, lower forms of life are and have been ever coming

[1] This section should ultimately be bound at the end of the several parts composing the work.

[2] According to Darwin, "all the living forms of life are the lineal descendants of those that lived long before the Cambrian epoch" ("Origin of Species," 6th ed., p. 428).

[3] "The Beginnings of Life," 1872, *Preface.*

into existence afresh, the doctrine of evolution is no longer stultified by their presence.

The recognition of such processes and their marvellous nature cannot fail, however, to teach us humility, in the face of our utter inability to explain them. Just as we cannot really explain why this or that saline compound, under similar conditions, invariably crystallises into some particular and well-known form, so are we even more hopelessly unable to understand why living matter, in some of its most elementary combinations, seems compelled now to take on the form of an Amœba, now of an Actinophrys, now of a Peranema or of a flagellate Monad. We may think and talk of "polarity of molecules," it is true, and try to find in such a notion some dim hints as to the causes that are in operation. Thus Herbert Spencer, while repudiating a belief in "spontaneous generation" and speaking of what he terms "physiological units," has written as follows:[1] "As certainly as molecules of alum have a form of equilibrium, the octahedron, into which they fall when the temperature of their solvent allows them to aggregate, so certainly must organic molecules of each kind, no matter how complex, have a form of equilibrium in which, when they aggregate, their complex forces are balanced—a form far less rigid and definite, for the reason that they have far less definite polarities, are far more unstable, and have their tendencies more easily modified by environing conditions."

We have in these words, undoubtedly, pregnant hints; and the transitions are so frequent, and seemingly so facile, between such simple forms of life as the flagellate Monad, the Amœba, the Actinophrys, and the Peranema, that one's thoughts naturally revert to known facts concerning allotropic and isomeric transformations of simpler kinds of matter, and to differences in the crystalline form assumed by various saline substances when aggregating under different conditions,[2] as affording the only sort of clue to the meaning of the rapid transitions so often occurring between these simple forms of life.

Proofs of the Doctrine of Evolution.

It is not only, however, that the proof of the actuality of such processes of Heterogenesis (as I have illustrated in this and shall further illustrate in future memoirs) removes one of the principal stumbling blocks in the way of the acceptance of the doctrine of evolution by accounting for the existence of lower organisms at the present day—it does still more, it furnishes perhaps the best and most direct proof of the doctrine of evolution itself. In Huxley's last public utterance on this

[1] "Principles of Biology," first edn., vol. ii. Appendix, p. 488.
[2] See art. "Dimorphism," Watts' "Dictionary of Chemistry."

subject he maintained that the actual proof of evolution rested upon palæontological evidence; that though the publication of Darwin's famous "Origin of Species" had brought about the general change of view in favour of this doctrine, yet the proof of the reality of evolution was independent of the truth of Darwin's views or those of others as to the modes in which evolution had been and was being achieved.[1]

Without questioning for a moment the dictum of Huxley as to the enormous importance of palæontological evidence in proof of the general doctrine of evolution, I would say that this evidence cannot compare in directness and in detail with that which is afforded by such processes of Heterogenesis as may be seen constantly taking place at the present day.

While palæontological evidence undoubtedly tends to prove the general doctrine of evolution, it affords little or no evidence as to the mode of origin of particular species.

Recent and most important evidence tends indeed to show unmistakably that even in regard to some higher forms of life the origin of new species may occur by sudden mutations rather than by the cumulation of minute progressive variations. Such sudden mutations must, however, be considered as sublimated forms of heterogenesis—such as are alone capable of occurring in comparatively high and complex organisms.

In a recent important work[2] de Vries, the Professor of Botany at Amsterdam, seems to have certainly demonstrated this point in regard to a plant named *Œnothera Lamarckiana*, by observations carried on during ten years upon 50,000 of the descendants of some of these plants placed by him in the botanical gardens of Amsterdam. Preliminary experiments made with this and a number of other plants showed it to be favourable for such observations. Of the 50,000 descendants of this plant, it was found that "about 49,200 were in no respect different from the original patriarchal *O. Lamarckiana*, showing no tendency towards gradual change in any special direction, but only the common small fluctuating 'variations' as regards size and appearance on either side of a normal—in fact, resembling in that respect other plants and animals which are left to themselves without being interfered with."

"Quite otherwise with the 800 other plants. None of these, although appearing spontaneously, could be said to be representatives of *O. Lamarckiana* from which they were descended. De Vries arranges them in seven distinct species, viz., 1 of *O. gigas*, 56 of *O. albida*, 350 of *O. oblonga*, 32 of *O. rubrinervis*, 158 of *O. Uanella*, 221 of *O. lata*, and 8 of *O. scintillans*."

[1] *Nature*, November 1, 1895.

[2] "Die Mutationstheorie," Ersten Band, Leipzig, 1901, a review of which by an able writer appeared in *Nature*, June 27, 1901, p. 208, and some quotations from which I subjoin.

Then again the representatives of these new species produced descendants the majority of which unmistakably belonged to the same species as itself. Some of the new species like *O. gigas* are very stable forms. Thus from the one specimen of this plant "were obtained 450 plants, all of which, with one exception, were *O. gigas*—the one exception not being a return to *O. Lamarckiana*, but belonging to a new variety." On the other hand, *O. scintillans* is "extremely unstable, *i.e.*, possesses the property of mutation to a high degree, a large proportion of its descendants belonging to other species, especially *O. oblonga* and *Lamarckiana* itself."

Referring to these highly important and convincing observations of de Vries the reviewer writing in *Nature* says, "a new period in the theories of the origin of species and of evolution has been inaugurated. . . . So far as his observations go new species appear *suddenly* by *mutation*, never as the outcome of a progressive variation.[1] . . . De Vries' experiments support the results arrived at by Scott and other palæontologists that there is no evidence in the successive strata of the earth of a gradual development of one species into another, and that everything points to small but sudden transitions."

There cannot be a doubt as to the very great importance of these observations of de Vries. They abundantly confirm the validity of scattered facts previously known, and open up, therefore, possibilities hitherto but little recognised as to a mode of origin of new species among higher plants and animals—that is, by processes which seem to carry on into these higher forms of life a minimised and modified form of those transmutations (far more startling in character and far more easily watched in their various phases) that occur so commonly among the lower vegetal and animal organisms with which we have been concerned in this memoir.

Referring to one of the most remarkable of the facts of this kind previously known I have elsewhere written as follows.[2]

"Again, as a proof that at rare intervals what appear to be totally distinct specific forms may arise from the embryo of a given species of animal, we may cite the most remarkable instance[3] of the appearance of the black-shouldered peacock (*Pavo nigripennis*) amongst Sir J. Trevelyan's flock composed entirely of the

[1] This must be considered to hold good only for these particular observations of de Vries. But in recent years a vast amount of evidence has been accumulated tending to prove that the origin of new species, both vegetal and animal, may be brought about by the accumulation of progressive variations, under the influence of altered external conditions. An excellent summary and reference to this new work is to be found in an article entitled "Recent Science," by Prince Kropotkin, in the *Nineteenth Century* for September, 1901.

[2] "Beginnings of Life," vol. ii., p. 598.

[3] Darwin, "Animals and Plants under Domestication," vol i., p. 290.

common species. The new form increased ' to the extinction of the previously existing breed,' and it is regarded by several of our best authorities as a distinct 'species': and yet this black-shouldered peacock has been known to have had a similar origin five times in England. There is no real reason, therefore, why such changes should not also at times occur with plants or animals living in the wild state. Mr. Murphy aptly remarks:[1] 'It may be true that we have no evidence of the origin of wild species in this way. But this is not a case in which negative evidence proves anything. We have never witnessed the origin of a wild species by any process whatever; and if a species were to come suddenly into being in the wild state, as the Ancon sheep did under domestication, how could you ascertain the fact? If the first of a newly-begotten species were found, the fact of its discovery would tell nothing as to its origin. . . . How is it possible, therefore, to gauge the amount of this kind of change which takes place amongst higher organisms, whereby new species may more or less abruptly appear upon the scene?' "

Persistent Types of Life.

Again, the present occurrence of Heterogenesis in these regions of the globe being established it may be regarded as a natural inference, from the doctrine of the "uniformity of natural phenomena," that the lowest forms of life of all kinds have been constantly having countless new origins thereon, both in time and in space; and these considerations may help us a little to understand why there should be what Huxley termed "persistent types" of life, not only among lowest organisms but of kinds even far above the lowest, the remains of which are to be met with in successive geological strata as well as in those whose formation has often been separated by vast periods of time.[2] It must be conceded that throughout all the life-evolving

[1] "Habit and Intelligence," vol. i., p. 344.

[2] Thus Huxley says (*Proceed. of Royal Instit.*, vol. iii., p. 151):—"Certain well-marked forms of living beings have existed through enormous epochs, surviving not only the changes in physical conditions, but persisting comparatively unaltered, while other forms of life have appeared and disappeared." He then cites numerous examples belonging both to the animal and to the vegetal worlds. Dr. Carpenter had also previously ("Intoduction to the Study of the Foraminifera," 1862, p. xi.) pointed out that "there is no evidence of any fundamental modification or advance in the Foraminiferous type from the palæozoic period to the present day." He says that similar types and similar varieties from those types are to be met with in geological formations existing as far back as the triassic rocks; and he therefore came to the usual conclusion that has hitherto been drawn from such facts—namely, that the forms of Foraminifera existing at the bottom of our oceans in the present day, are the lineal descendants of those similar forms which lived ages and ages ago in the oceans existing when the triassic rocks were being formed. In regard moreover to some higher forms of life Huxley spoke as follows:—"Turning to the

periods of the history of our globe the essential mode and progress of organisation has been, in the main, the same. In the light afforded, therefore, by the recognition of the occurrence of Heterogenesis, persistence of types is to be expected, seeing that the living things which have been constantly arising by such processes have been the immediate products of ever acting material properties or natural laws—the same in all times, however much or little the environing conditions may have varied from age to age. All the lowest stages of animal and of vegetal life must, therefore, have been repeated over and over again in successive geological periods—though up to what grades of organisation the occurrence of such repetitions has been frequent must remain altogether uncertain.

That a great amount of similarity should exist between the earlier forms of organisms that appeared at Silurian periods and those which exist at the present day is not so much to be wondered at when we recollect that the starting point, or primordial constitution of living matter, has ever been generically the same, and that the internal determining causes of change, represented by 'organic polarity,' have been constant. "Looked at broadly, it is not contrary to what we might expect if we find that the most marked and long-continued similarity exists between some of the lowest forms of life preserved in the fossil state, which being tenants of deep seas and oceans, have all along been exposed to almost similar conditions."[1] Thus, as I would still submit, it seems not unreasonable to hold that the 'persistence' in geological formations, widely separated in time, of "the lower invertebrate types is much more explicable on the assumption of successive evolutions of more or less similar forms from similar starting points under the influence of like conditions, than on the assumption that such changeable forms should have reproduced their like without any very appreciable alteration through such vast and unrealisable epochs of time."[2]

Wide Distribution over the Earth of Similar Lowest Types of Life.

Considerations of a like kind may also account for the widespread distribution over almost all parts of the globe at the present

Mollusca, the genera *Crania*, *Discnia*, and *Lingula* have persisted from the Silurian epoch to the present day with so little change that competent malacologists are sometimes puzzled to distinguish the ancient from the modern species." Numerous other examples of this persistence of type have been referred to by Professor Poulton in his address to the British Association in 1896 as president of the Zoological Section.

[1] "The Beginnings of Life," vol. ii., p. 617.

[2] *Loc. cit.*, p. 616.

day of multitudes of the same kinds of lowest vegetal and animal organisms. Such facts seem to show that the intrinsic properties of the more or less similar matter from which they have been derived have more to do with the forms and structures of these lower organisms than any difference in the conditions under which they have been born. It may be, therefore, that the essential similarity undoubtedly obtaining among so many of the simpler forms of life existing in different parts of the earth's surface at the present day is due to the fact that the elementary forms of vegetal and animal matter, from which so many of them have been derived by processes of heterogenesis, have also an essentially similar nature in these various regions. Under the dominating influence of 'organic polarity' the several forms thus produced unfold, therefore, more or less immediately into such and such simple organisms of common type, according to the particular molecular composition of the altered elementary matrices from which they arise.

I say dominating influence of 'organic polarity,' because it is clear that heredity must be a non-existent factor for these chance products originating by heterogenesis; and because, as I believe, and long ago maintained, the influence of 'natural selection' as a modifying agent is probably extremely slight among all the lowest forms of animal and vegetal life—even though Darwin was inclined to make no such limitation as to the sphere of its influence.

Then, again, I would point out that such views as I have above expressed would diminish to an enormous extent many of the difficulties at present existing in regard to the geographical distribution of plants and animals. Darwin, with his usual candour, says[1]: "Undoubtedly there are many cases of extreme difficulty in understanding how the same species could possibly have migrated from some one point to the several distant and isolated points where now found." He then goes on to say: "Nevertheless the simplicity of the view that each species was first produced within a single region captivates the mind. He who rejects it, rejects the *vera causa* of ordinary generation with subsequent migration, and calls in the agency of a miracle." Of course in speaking thus he was referring, as an alternative, to the obsolete view, which he so successfully combated, of the supposed special Creation of species in single centres. But so far from appealing to miracle, I appeal to the "uniformity of natural phenomena" in space and in time.

[1] *Loc. cit.*, p. 320.

The Question of the Time Needful for the Evolution of all the Forms of Life that have appeared upon the Earth.

Again, the rapidity with which lower forms of life are produced by heterogenesis, seeing that, as I have shown, a few days may take the place of the "enormous period of time" previously postulated for the evolution even of such organisms as ciliated Infusoria, may not be without influence in bringing rather more into harmony, than is at present the case, the views entertained by biologists and geologists respectively as to the probable duration of life upon our globe.

The actual age of the globe, and also the time that must be supposed to have elapsed since the first appearance of living things upon its surface, has given rise to a considerable amount of discussion since Sir William Thomson (now Lord Kelvin) in 1862 first endeavoured to show in a paper [1] on "The Secular Cooling of the Earth," that great limitations had to be put upon the enormous demands for time made by Sir Chas. Lyell and his immediate followers in accounting for all the series of changes on the surface of the earth that come within the ken of the geologist.

The expression of Lord Kelvin's views in this paper for a time caused some consternation among both geologists and biologists. He returned to the subject in other communications in 1865, in 1868, and again in 1897, arguing from certain physical and astronomical data. His views as to the amount of time that could be conceded as possible for the entire age of the earth has varied in these different communications, though his tendency has been to limit it more and more, till in his latest utterance on this subject [2] he put it as "more than twenty and less than forty millions of years, and probably much nearer twenty than forty."

His views and reasonings have been criticised by other physicists, and especially by Professors Perry and George Darwin, who have attempted to show, with much success, the uncertain nature of the data and assumptions upon which Lord Kelvin's conclusions have been founded.

Moreover, the time limits assigned by him have been considered altogether inadequate by geologists and biologists alike. The most authoritative demurrer on the former side may be found in Sir Archibald Geikie's address[3] to the Geological Section of the British Association in 1899. He considered that nothing short of 100,000,000 years would be adequate "as the period

[1] *Trans. of Roy. Soc. Edin.* vol. xxiii.
[2] *Phil. Mag.*, January, 1899.
[3] *Nature*, September 21, 1899.

within which the history of life upon the planet must be comprised "—and of course very much more for the total life of the planet. On the other hand Professor Poulton, three years previously, in his address[1] to the Zoological Section of the British Association, had dealt in an elaborate manner with the problem from the biological standpoint. He seemed to consider that a period at the very least four or five times as long as that just mentioned as satisfying the geologists would be needed to account for the evolution of all the forms of life upon the globe—that is, those that appeared anterior to the Cambrian epoch, those whose remains have been preserved in the fossil state together with their unpreserved contemporaries, as well as those at present existing on the surface of the earth. The pre-Cambrian period, in his estimation, may have been three or four times as prolonged as the subsequent ages during which the whole stratified crust of the globe has been laid down and all the forms of life known to us have been evolved.

This view as to the probable vast duration of the pre-Cambrian ages is shared by geologists generally, and is also in accord with views expressed by such leading evolutionists as Darwin, Herbert Spencer and Huxley.

But, as I have said, the first announcement of Lord Kelvin's conclusions undoubtedly came as a shock to biologists and geologists alike. Thus Darwin, writing to Wallace on April 14, 1869, said: "Thomson's views of the recent age of the world have been for some time one of my sorest troubles." For even the widest limit that Lord Kelvin at that time was prepared to concede for the whole age of the world was altogether at variance with what would be required for the mere duration of life upon its surface in accordance with Darwin's views as to the means by which new species had been evolved, and his supposition of a single starting point for living matter at some one particular time, and in some one particular place on the surface of the earth.[2]

Another of the principal reasons that made it necessary for Darwin to make extremely large demands upon time is to be found in his view that low forms of life change or become modified less quickly than the higher forms.[3] This same doctrine was also most strongly enforced by Poulton in the before-mentioned address. He said,[4] "undoubtedly a study of all the available evidence points very strongly to the conclusion that in

[1] *Nature*, September 24, 1896.

[2] "Origin of Species," sixth ed., p. 429.

[3] *Loc. cit.*, p. 346.

[4] *Loc. cit.*, p. 506.

the lower grade, sub-grades, and Phyla of the animal kingdom, evolution has been extremely slow as compared with that in the higher."

But these views as to the rate of change in lower organisms are based upon the most questionable data. It is obvious that Poulton was in the main influenced by the facts known concerning "persistent types" among the lower forms of life existing in geological strata from the Cambrian and Silurian periods onwards. He, indeed, distinctly intimates that the longer the persistence through geologic ages of any given type of life the greater would have been the time needful for its origination.[1]

What I have already said concerning "persistent types," however, will have shown that a totally different interpretation may be put upon such facts when once the doctrine of heterogenesis is admitted. It certainly would no longer be needful to assume, with Darwin and with Poulton, that the rate of change is slow among low as compared with higher forms of life. Such a conclusion is directly opposed to a large mass of other evidence, and is, I believe, absolutely the reverse of the truth.

Then again, if instead of believing with Darwin that "all the living forms of life are the *lineal* descendants of those that lived long before the Cambrian epoch," and that "all the organic beings which have ever lived on this earth may be descended from some one primordial form," it should be admitted that life originally started from multitudes of centres (as the uniformity of natural phenomena would demand); that from the earliest stages of the earth's history up to the present time new starting points of simplest forms (by heterogenesis) have been ever taking place all over the surface of the earth, we may see, not only how many of the facts concerning "persistent types" may be explained, but also how the time needed for the whole evolution of life upon the globe may have been far less prolonged than biologists have hitherto supposed.

[1] *Loc. cit.*, p. 509.

APPENDIX II.

NOTE ON THE TRANSFORMATION IN THE COURSE OF THREE OR FOUR DAYS OF THE ENTIRE CONTENTS OF THE EGG OF HYDATINA SENTA INTO A LARGE CILIATED INFUSORIUM BELONGING TO THE GENUS OTOSTOMA. By H. CHARLTON BASTIAN, M.D., F.R.S.

*** The Note which follows announcing an increase of 'natural knowledge' of the greatest importance for biological science was sent to one of the Secretaries of the Royal Society on January 16, 1902, with the request that it might be brought before the Society at an early date, and that, in view of the startling nature of the announcement, I might be allowed to give a microscopical demonstration, with living specimens, of some of the phases of the transformation in question, either before or after the meeting.

I was told that the Note must be referred to the Sectional Committee on Zoology; and it was so referred. At the expiration of a month, having heard nothing more on the subject, I wrote to the acting Chairman of that Committee (the actual chairman being abroad) saying that if a short paper from a Fellow of the Society had to be submitted to a Committee I presumed it could only be in regard to one or other of two questions. Were the announcements in the papers new, or were they true? In regard to the first point there seemed to be no room for doubt; and in regard to the second I asked how the Committee was to come to a decision without the examination of specimens—which I was prepared to show them.

As a result on February 19 the acting Chairman of the Committee came to my house, but he had to hurry away in order to catch a train to Cambridge, before he had seen much more than one half of the photographs, together with microscopical specimens, living and preserved, which I had prepared for his examination. Some correspondence followed, and at his request I sent him a few specimens. It soon became plain to me from statements made in his letters, now in my possession, that however accomplished this gentleman might be in other directions, he had very little practical acquaintance either with Rotifers or with Ciliates, and was not always able to distinguish a Rotifer's egg from an encysted Ciliate. He declined my invitation to come again and examine living specimens as he seemed to think the most important thing to be done was to submit the eggs to histological examination—and no further communication passed between us.

Meanwhile another influential member of the Sectional Committee to which my paper had been referred, Professor Ray Lankester, refused point blank, on two occasions, to look at the specimens or photographs illustrating the transformation in question—even though on the last of these occasions he was standing within three yards of them, and in the Library of the Royal Society.

Not one of the other members of the Committee sought to see my specimens, or made any enquiry whatever in regard to the matter.

This was the action—or rather inaction—of the Sectional Committee to which my Note had been referred. They were content to base their verdict

upon *à priori* considerations as to what seemed to them possible or impossible. Accordingly, early in April I was officially informed that the Committee was of the opinion that "the paper should not be accepted unless the author can provide a full and definite histological proof of the transformation of the Rotifers' eggs into Ciliates; accompanied by a detailed history of the metamorphosis."

This was their answer to my wish to make known to biologists generally the simple means by which they might witness and study for themselves, in any way they might choose, the stages of the transformation in question. But it was decided that no preliminary Note was to be published to make these means known; that biologists should be kept in ignorance of them, so far as the Royal Society was concerned, until I could produce a complete and illustrated memoir, with all histological details, and, of course, submit it again to this Committee that they might judge of its fitness, or not, for publication in the *Philosophical Transactions*.

The Note contains, as I maintain, abundant evidence testifying to the reality of the allegations made, as well as simple details by means of which others might easily repeat my observations. But no, that would not do for this Committee; they would take no trouble themselves either to observe or exercise their reason on the statements made. They bade me undertake a prolonged research of a kind which, with my experience of the objects that would have to be employed, could not, I believe, be carried to a successful issue; and which would, moreover, have to be prefaced by a complete histological study of the normal development of the Hydatina egg.

In the interval, looking to all the delay that was occurring, and realising from the behaviour of the Committee—and especially that of the influential member of it who refused even to look at my specimens—that there was no chance of my Note being published by the Royal Society, I had, before the announcement of their decision reached me, caused it to be translated into French and German, and had sent copies of it simultaneously to the Academies of Science of Paris, Rome and Berlin.

What happened was this. The Note was submitted to the Académie des Sciences of Paris on April 1, and was then referred for consideration to Professors Ranvier, Perrier and Delage. Up to the present (June 10) I have heard nothing from them, although soon after the nomination of these referees I sent them a set of thirteen photographs illustrating different stages of the transformation in question.

On April 3 the Note was submitted to the Königl. Akademie der Wissenschaften of Berlin, and I was subsequently informed by one of the Secretaries that it had been consigned to their Archives. I was told it had been laid before a Committee of experts, and that they were of opinion the paper was not suitable for publication, as my observations "were only a repetition of mistakes that other workers in the same line had previously made." This again was a mere *à priori* judgment apart from any enquiry.

In Rome the paper was not even laid before the R. Accademia dei Lincei. The Secretary took it upon himself to submit it to the judgment of a single expert, and his opinion not being favourable to its publication, inasmuch as my "conclusions were contrary to actual scientific knowledge," the communication was politely returned to me.

Of course I fully recognise that if in sending my Note to one or other of these foreign Academies I could have said that it was to be communicated simultaneously, at a particular meeting, to the Royal Society the result might have been different. Still what has happened illustrates thoroughly the present point of view of the scientific world in regard to Heterogenesis, and the consequent dead weight of preconception with which I have to contend.

I still venture to think, however, that about as much real evidence as could possibly be expected is to be found in this preliminary Note, and that if the

publication of such an addition to 'natural knowledge,' as it was destined to make known to working biologists and others, was declined by the Royal Society and the other Academics above mentioned it can only be ascribed to two facts, namely, (1) to the extreme importance combined with the seeming improbability of the phenomena announced; and (2) to the existence, even at the present day, in some individuals of a regrettable spirit of intolerance for new truths out of accord with their preconceptions—intolerance so strong as to prevent an impartial consideration of evidence adduced.

After a few verbal alterations had been made in the Note as sent to the Royal Society in January, this, together with an Addendum, discussing the only two possible sources of fallacy, which was forwarded to the Society early in March, was translated for transmission to the foreign Academies from the text here reproduced.

Having recently witnessed on very many occasions the stages of this remarkable transformation of the contents of a Rotifer's egg into a Ciliated Infusorium, I am desirous of acquainting the Académie des Sciences with the simple procedure needful to enable zoologists to study for themselves the series of changes leading to a result which most of them will probably deem incredible until they have actually investigated the changes for themselves.

All that is necessary is to procure a good stock of these large Rotifers by placing some surface mud, having a coating of Euglenæ, from a ditch in which they are known to exist into a glass bowl, and to pour thereon water to a depth of about four inches. In the course of two or three days (with a temperature of 16° C.), if the Hydatinæ are abundant, a good crop of their large eggs will be seen at the surface of the fluid where it is in contact with the glass.

By the aid of a scalpel passed along their track for a short distance groups of 20 to 30 eggs may be taken up at one time, and gently pressed off the edge of the blade into a small, white stone pot full of water. Some of such small masses of eggs (mixed perhaps with a few Euglenæ) will float, and others will sink. After seven or eight of these masses of eggs have been gathered and deposited, the cover should be placed upon the pot so as to cut off from the eggs all light rays, both visible and invisible.

If the supply of eggs will admit of it two other pots should be similarly charged; but if there are not enough eggs for this purpose, the two other pots should be charged on successive days with fresh batches of eggs. The larger the supplies of fresh Hydatina eggs the more convincing will be the result.

When the pots have remained covered and undisturbed for thirty-six hours at a temperature of about 17° C., one of them may be opened, and some of the small masses of eggs from the bottom of the pot should be taken up with a tiny pipette and placed in a drop of water on a microscope slip. Before covering the specimens a minute fragment of a cover-glass should be

placed at each side of the drop of water, so as to protect the delicate eggs from undue pressure.

On examination by a low power of the microscope it will be seen that there are many empty egg-cases, that within some eggs there are malformed embryo Hydatinæ in different stages of development, while within the remaining eggs the contents will be wholly different, consisting of an aggregate of minute pellucid spheres or vesicles each containing a few granules, together with a variable amount of granules interspersed among the vesicles.

If the cover should be again placed upon this pot with a view to the examination of other portions of its contents twenty-four or thirty-six hours later, and this examination is made, it will be found that no further advance has taken place—that the eggs previously in the vesicular condition still remain in this stage, and are in fact no further advanced than some of their fellows were when previously examined.

I have found on many occasions that the opening of a pot at an early stage of the transformation—even for only four or five minutes—arrests the whole process of change. It was for this reason that I advised three separate pots to be charged, so that their contents might be examined at different periods.

When, however, a second pot is opened two and a half or three days after the eggs have been placed therein, and portions of its contents are examined in the same way, a larger proportion of empty egg-cases will be seen. There may be very few or even no malformed Rotifers still within the eggs ; and in others, instead of the motionless vesicular contents previously seen, large Ciliates may be found slowly revolving within unruptured egg-cases, or, under the influence of the light, struggling out, and swimming away with rapid movements, partly of rotation. Some of the Infusoria before they emerge undergo segmentation into two, four, or rarely, even into eight smaller Ciliates.

The large undivided Infusoria have their bodies densely packed with large corpuscles (modified representatives of the vesicles of an earlier stage) ; they have a large contractile vesicle, and also a large elongated nucleus. They possess the characteristic ear-shaped mouth indicated by the name Otostoma, and cilia are distributed all over the body in longitudinal lines, so as to give the appearance of a delicate longitudinal striation.

In the event of the Ciliates being not yet fully developed at the time that the second pot is opened, we have the third pot whose contents we can investigate slightly later.

As a control experiment it will be well at the time that the pots are charged to place two or three batches of the eggs with some of the same water into a watch glass, which is left exposed to light; and at the expiration of three or four days, as well as at later periods, to search among its contents for any of the same

large Ciliates, and also for any eggs in the intermediate vesicular stage above referred to. I can confidently predict that this search will prove unsuccessful.

In taking batches of eggs, in the manner I have indicated, to be placed in the pots, they will necessarily be of different ages. Some will have already begun to develop into Rotifers, and these, under the altogether unnatural conditions to which they are subjected in the dark pots, become more or less malformed as development proceeds. Others that have been quite recently laid will not have begun to develop, and it is these latter eggs apparently which, under the cutting off not only of ordinary light but probably of some invisible light rays, become transformed into Ciliated Infusoria. Cutting off ordinary light rays alone from the eggs, by placing them in a small covered glass dish shut up in a cupboard or box and maintained at the same temperature as before, seemed at first not to lead to similar results, though I have since ascertained that the transformation will occur under such conditions, though only after the lapse of about nine days. It looks, therefore, as if the stoppage of some invisible rays, capable of passing through wood but not through stone, notably hastens the process.

Then, again, it has seemed to me that a larger proportion of those eggs taken from the bottom of the pots yields these large Ciliates than of those remaining at the surface of the fluid. Hence I fill the pots full of water so as to leave as little air as possible even for those floating on the surface.

Briefly enumerated, the stages of the transformation are these:—

(1) A freshly-laid Hydatina egg; (2) partial rearrangement of its contents on the way to the formation of a mass of small spheres or vesicles; (3) the conversion of the whole of the egg-substance into a mass of spherical vesicles of varying sizes; (4) changes in the constitution of the vesicles and the appearance of more granules between them; (5) the formation of the embryo Otostoma within an almost invisible hyaline membrane; (6) the development of Cilia, the slow rotation of the embryo within this envelope, and the occasional appearance of a contractile vesicle; (7) the bursting of the hyaline envelope, with freer play of Cilia and more rapid movements within the egg-case; (8) in some instances fission into two, four, or more, active segments; (9) rupture of the Rotifer egg-case, and the appearance of its contents in the form of a very large and rapidly moving Ciliated Infusorium.

[*An* Addendum *to the foregoing Note, sent to the Royal Society early in March last.*]

However improbable this transformation may seem to those who have not studied the changes for themselves, the possibilities of error are still more improbable.

The only conceivable sources of error would seem to be these two: (*a*) That each fresh Hydatina egg seeming to undergo the transformation in question has become infected by some single immature form of the Otostoma; or (*b*) that what I take to be Hydatina eggs have really been Encysted Otostomas.

But the evidence against each of these interpretations is in reality overwhelming, as may be seen from the following statements:—

Evidence against the Infection Hypothesis.

(1) No such infection by a Ciliate is known to science.

(2) No 'spores' of Ciliates are known to exist.

(3) If a young Otostoma had been able to penetrate the egg it would be seen there, gradually growing and devouring the contents of the egg, till only the Otostoma itself remained completely filling the egg-case. But nothing of this kind is to be seen.

(4) On the contrary, the various stages of change, from the evenly granular condition of the fresh Hydatina egg to the revolving Otostoma, have been made out and photographed.

(5) The stages of change from the fresh egg to the formation of the young Hydatina developing under normal conditions are wholly different.

(6) If young Otostomata were present with the Hydatina eggs then they, as well as the adult forms, ought to be discoverable before the batches of Hydatina eggs are placed in the pots; which is not the case.

(7) And, again, if Otostomata were present and capable of penetrating Hydatina eggs, the transformation I have described should occur also when batches of the fresh Rotifer eggs are placed within a watch glass exposed to ordinary daylight. But under these conditions no such transformation occurs.

(8) Yet when similar batches of thirty to forty Hydatina eggs are placed as I have directed, in a dark pot, from five to ten specimens of this otherwise extremely rare Ciliate may be found in each such batch of eggs, at the expiration of three to four days.

Evidence against the Encysted Ciliate Hypothesis.

(1) There is the extreme improbability of an encysted Ciliate being mistaken for a Hydatina egg, or *vice versâ*, by anyone familiar with such objects.

(2) The free Hydatina egg, for identification, can be compared with others within the bodies of parent Rotifers, and, again, Hydatinæ may be seen forming within, and escaping from, the extremely common free eggs.

(3) An encysted Ciliate of the size of the Hydatina egg is extremely rare; and the alleged changes from the evenly granular, newly-laid Hydatina egg on to the formation of the Otostoma, are of a kind wholly different from anything that occurs in a previously active Ciliate which has become encysted.

(4) But no Otostomata either free or encysted are to be found among the batches of fresh Hydatina eggs placed in the pots.

(5) The egg-case left by a young Rotifer after its escape is exactly similar in size, in thickness, and in its histological characters to that which is left by the Otostoma when it escapes.

(6) Eggs have been seen within a dead Hydatina going through their development into embryo Rotifers; while in other dead Hydatinæ, taken from a pot, eggs have been seen, as my photographs show, in one of the intermediate vesicular stages such as always occur during the development of an Otostoma from a Hydatina egg.

(7) A full-sized living Otostoma has been found within the unruptured integuments of a dead Hydatina taken from one of the pots.

A careful consideration of the above-mentioned facts must make it clear that neither of the two possible sources of error can be shown to have any real existence; and I can only repeat, without hesitation, that the facts are as I have represented them to be, and that it is the entire substance of the fresh Hydatina egg which, under unnatural conditions, becomes converted into a great Otostoma.

APPENDIX III.

ON THE GREAT IMPORTANCE FROM THE POINT OF VIEW OF MEDICAL SCIENCE OF THE PROOF THAT BACTERIA AND THEIR ALLIES ARE CAPABLE OF ARISING DE NOVO.

The intimate relations that have been proved to exist between microorganisms and so many of the contagious or communicable diseases, and the discovery that in many cases the organisms in question act as the veritable contagia by means of which such diseases are spread from person to person, have exercised such an enormous influence over the minds of medical men that they have led to the almost universal establishment of ultra-contagionist views in regard to all these diseases. Just, it is thought, as organisms are propagated only and do not arise *de novo* so the reigning doctrine in medicine has been for some time, and still is, that contagious diseases are propagated only and never arise *de novo*. The breaking down of the prejudice in regard to organisms by showing that they can originate independently of pre-existing microorganisms of like kind would of necessity exert a powerful influence over medical doctrines and would pave the way for the admission that contagious diseases may also arise *de novo* instead of being only disseminated by contagion. Short of a proof of this kind there seems less chance of any such widening of doctrine being brought about.

To show the kind of feeling that exists I may recall the fact that one of the most distinguished physicians in this country not very long since said:[1] "If I can trace contagion in a very large number of the so-called specific diseases I consider it more reasonable to assume contagion in the minority than look about for another cause." And he went on to say that, as many of these diseases are associated with the growth and multiplication of "living specific organisms" a belief in the *de novo* origin of these contagious diseases would "imply also a belief in spontaneous generation." This latter notion is undoubtedly very common and has been one of the principal causes that has stood in the way of a belief in the possibility of the *de novo* origin of a contagious disease. On this account, therefore, the proof of the heterogenetic origin of Bacteria becomes a matter of very great

[1] Letter from Sir Samuel Wilks, *British Medical Journal*, December 23, 1893.

importance for medical science. Still, the conclusion above drawn, notwithstanding its prevalence and great influence, does not of necessity follow, as I shall hope to show.

We all know that common Bacilli and Micrococci are constantly making their entry into the body through the intestinal and the respiratory mucous membranes and thence are gaining access to the lymphatic system. So that for the origin of this or that specific disease it may not be at all necessary that a *de novo* origin of microorganisms should take place. Under the influence of unhealthy local or general conditions the common microorganisms thus entering into the body may possibly be made to take on new properties and be, in fact, converted into one or other of the so-called "specific" or "pathogenic" microorganisms.

It is needless for me to cite in support of this latter possibility the vast array of facts now known concerning the variations and interchangeability of form that may be brought about in these microorganisms by changes in the media and conditions to which they are subjected, and the still more important variations in function and in the chemical processes associated with their growth and multiplication that may be similarly induced. This is so notoriously the case that many writers, such as Billroth, Nägeli, Warming, Cienkowski, Ray Lankester, Zopf, and others have regarded these microorganisms as mere developmental phases or varieties, modified by external conditions, of one and the same, or but a very few distinct species. Many facts of importance in this relation in connection with pathogenic Bacteria and their possible derivation from non-pathogenic forms are matters of common knowledge.

There is one case, however, so pertinent to the present inquiry and of such great importance in itself that some details will prove most useful. I allude to our present knowledge concerning certain experimentally produced diseases in lower animals included under the name *Septicæmia.* Two of these forms of septicæmia have been investigated experimentally with the greatest care.[1] One of them, known as "Davaine's septicæmia," may be originated in a previously healthy animal by injecting two or three drops of putrid blood (bullock's or that of any other animal) into the subcutaneous tissue of a rabbit. The animal dies in from twenty-three to twenty-five hours, Bacilli in myriads and of a distinctive kind existing, even during life, in its blood. This constitutes the origin of a disease which proves to be

[1] See "Report on Experimental Investigations on the Intimate Nature of the Contagium in Certain Acute Infective Diseases," by G. F. Dowdeswell, *British Medical Journal*, July 19, 1884, pp. 101-8.

contagious, and so much so that it can be propagated from animal to animal by even the millionth part of a drop of blood, and thus on indefinitely. The other form is known as "Pasteur's septicæmia" and it is produced at will in this fashion. Let two or three drops of putrid bullock's blood from the same stock be this time injected into the peritoneal cavity of a rabbit rather than into its subcutaneous tissue, and now a different form of disease is established, though one which is also contagious and equally constant in its characters. In this case the animal does not die so rapidly, and while Bacilli swarm in the fluids of the peritoneal cavity within twenty-four hours they are not to be found at the time of death in the blood, though they appear there and throughout the body in the course of a very few hours after death. Another difference between these two varieties of septicæmia is that though the latter form of the disease is also contagious and capable of being propagated indefinitely by the inoculation of a minute quantity of the peritoneal fluid, yet this fluid contains a contagium which is nothing like so virulent as that contained in the blood in "Davaine's septicæmia." Instead of one millionth of a drop, which is adequate for contagion in this latter case, it is found that about a drop of the infecting peritoneal fluid is needed in the case of "Pasteur's septicæmia."

But now another and even more important point has to be mentioned. It is this. We are told that absolutely no difference can be detected between "Pasteur's septicæmia" and that which is initiated after the manner of Burdon Sanderson by injecting a small quantity of a germ-free chemical irritant into the peritoneal cavity or into the subcutaneous tissue of a rabbit. Difference in the site of introduction of the mere chemical irritant produces no difference in the disease. Its action in either situation is to set up a most virulent inflammation, the fluids of which speedily teem with Bacilli, and in every particular the malady so induced has been shown by other pathologists exactly to resemble "Pasteur's septicæmia." Burdon Sanderson's words concerning the actual *de novo* production at will of this contagious disease are as follows:[1] "If a few drops of a previously boiled and cooled dilute solution of ammonia are injected underneath the skin of a guinea-pig a diffuse inflammation is produced, the exudation liquid of which is found after twenty-four hours to be charged with Bacteria. . . . . Other chemical agents will lead to the same results and *always under conditions which preclude the possibility of the introduction of any infecting matter from without.*" Elsewhere[2] the same investigator referred to

[1] *Transactions of the Pathological Society*, 1872, pp. 306-8. (The Italics are not in the original.)

[2] *Transactions of the Royal Medical and Chirurgical Society*, 1873, p. 365.

experiments which were made about the same time in order to throw light upon the cause of the appearance of Bacteria in certain peritoneal exudations and to ascertain whether or not their presence was to be considered as "a mere result of the intensity of the peritonitis." He says: "To determine this, experiments were made during the following month (May, 1871) which consisted in inducing intense peritonitis by the injection not of exudation liquids but of chemical irritants, particularly dilute ammonia and concentrated solution of iodine in hydriodic acid. As regards the ammonia, precautions were taken to guard against contamination by boiling and cooling the liquids as well as the implements to be used immediately before injection. In the case of the solution of iodine this was, of course, unnecessary. In every instance it was found that the exudation liquids, collected from twenty-four to forty-eight hours after injection, were charged with Bacteria, whence it appeared probable that the existence of these organisms was dependent, not on the nature of the exciting liquid by which the inflammation was induced, but *on the intensity of the inflammation itself*."

The organisms in the cases where germ-free chemical irritants have been employed have therefore come from the previously healthy body of the animal experimented upon—either by way of heterogenesis or by the waking up of previously "latent germs" of common microorganisms, instead of being, as in other experiments, modified descendants of the common putrefactive organisms contained in the bullock's blood. But the point of importance is that in either case, under the influence of local inflammatory processes of great intensity, such common organisms have been converted into specific or "pathogenic" microorganisms, capable henceforth of preserving their specific characters and of "breeding true" in suitable media.

Dowdeswell, who has many times repeated these experiments for the production of both varieties of septicæmia, points out that the form named after Pasteur corresponds with what Koch has termed "malignant œdema," and that the bacillus which characterises it is an extremely common form often found in the outside world. Still, when the germ-free chemical irritants are used he declares his belief that infection from without is precluded and that the Bacilli "originated from within the animal organism."[1] In regard to the production of the form named after Davaine it appears that putrid blood sometimes fails, especially in winter. Some particular stage of the putrefactive process seems necessary. Nothing more than this is proved, though Dowdeswell assumes, without adducing a vestige of proof, that the organism characterising this form of the disease

[1] *Loc. cit.*, p. 104.

in some way gets into the putrefying blood owing to "atmospheric contamination." But this is a mere unsupported guess. The evidence tends to show that it is one of the many forms developed at a certain stage in the putrefying blood which is capable of infecting the system through the subcutaneous tissue but not when introduced into the peritoneal cavity. This Bacillus of Davaine's septicæmia is, according to Dowdeswell, a so-called "specific organism" whose characters he has minutely described[1]; and it is not known to exist in the outside world apart from putrid blood in certain stages of change.

This production of two different forms of septicæmia by the inoculation of some of the same putrid material into different sites is a matter of the greatest importance. The putrid blood under the skin gives rise to one form of specific microorganism and contagious disease, while two or three drops of the same putrid blood introduced into the peritoneal cavity of a similar animal give rise to swarms of a different organism and the development of another contagious affection. The differences in the inflammatory processes in the two situations are capable, that is, of transforming some common microorganisms into two quite different specific bacilli; while the germ-free chemical irritants, with even more certainty, give rise to one and the same contagious affection whichever may be the site of their introduction.

These are all facts the importance of which can scarcely be over-estimated. They afford a sort of beacon light capable of illuminating the obscurity surrounding the origin of many other contagious diseases. A few examples will suffice to show the mode in which they may prove helpful in overcoming difficulties which are commonly thought to stand in the way of a belief in the *de novo* origin of other of these contagious diseases.

In *Typhoid Fever* we have one of the commonest of contagious affections about the possibility of whose *de novo* origin the greatest difference of opinion has long existed, though of late the ultra-contagionist view has been decidedly gaining ground here as in other directions. But there are, perhaps, some still who, while admitting contagion and the common spread of the disease through contaminated water or milk, or by other agencies, would be inclined to agree with Rodet and Roux that this disease may originate *de novo* and that the typhoid Bacillus of Eberth is merely an altered and virulent form of the common Bacillus of the colon.[2] The researches of

[1] *Journal of the Royal Microscopical Society*, 1882, vol. ii., p. 310, and the *Quarterly Journal of Microscopical Science*, 1882, p. 66.

[2] As to the degree of the relationship between these forms and the frequent difficulty in distinguishing one from the other, see Crookshank's "Bacteriology and Infective Diseases," Fourth Edition, 1896, pp. 344-46.

these observers have tended to show that this latter Bacillus, which commonly exists in the human intestine without harmful results, can become highly virulent and infective, under certain conditions, when introduced into water. Hence they conclude that not only typhoid dejections but simple fæcal pollution of water may produce typhoid fever in those who drink it. This is undoubtedly a very important point and one which hitherto has not been adequately taken into account by those who have attempted, as they thought, to trace the source of contagion in many cases of typhoid fever. Because there has been pollution of a water source by a man suffering from diarrhœa, and typhoid has been produced in persons drinking such water, it must not be assumed without proof that the man who polluted the water was suffering from typhoid fever. As bearing upon this view of Rodet and Roux it is interesting to note that typical enteric lesions in the small intestine have been artificially induced in lower animals by R. Row[1] of Bombay by intoxicating them with the products of the Bacillus coli communis; and that the lesions thus produced have been even more marked than when similar animals have been intoxicated with the products of the more specialised Bacillus of typhoid fever—though in the latter case death was produced with more pronounced general symptoms and also more rapidly than in the former case.

While one mode of origin of the disease may be brought about in the manner indicated by Rodet and Roux (that is, in a manner only too likely to be ascribed to a spread of the disease by water-borne contagia), it is well known that another quite different mode of origin of the disease was advocated by Murchison in his celebrated work on "Continued Fevers."[2] He believed that the toxic cause of typhoid fever might originate in pent-up decomposing fæcal matter, and that in these cases the mode of entry of the poison into the system was through the air rather than by means of fluids taken into the alimentary canal.

Others, again, hold views closely related to this. Instead of supposing that the general health is lowered and the system poisoned by breathing the emanations from choked drains and cess-pits (causes to which isolated or small groups of cases of typhoid fever often seem traceable), they lay stress upon wide-spread pollutions of the soil beneath houses and upon variations in the height of ground water of such a kind as to facilitate the entry of emanations from such soil into houses, and the production thereby of slowly poisonous effects upon many persons

[1] *Transactions of the Bombay Medical and Physical Society*, vol. iv., No. 5.

[2] "The Continued Fevers of Great Britain," 1862, pp. 437-456.

simultaneously. They would thus account for the endemic and epidemic visitations of typhoid fever in particular towns and for their special autumnal prevalence. Speaking on this latter subject in a lecture on "Some Points in the Etiology of Typhoid Fever," Sir Charles Cameron,[1] the medical officer of health of Dublin, said: "Localised outbreaks of typhoid fever can frequently be directly traced to the use of a particular supply of polluted water or milk, but the widespread epidemics of this disease, and even its persistent occurrence in so many towns, must be due to some other cause or causes. For example, in Dublin it was epidemic in 1891-92, and in 1889 it appeared in all parts of the city and adjacent districts." These epidemics, Sir Charles Cameron feels assured, could not be traced to contamination of the water-supply, for in his view "there are few cities in the world with such good water as Dublin fortunately possesses." On the other hand, there is very poor natural drainage owing to the low-lying situation of the city, and the soil in very many parts is foul and saturated with decomposing organic fluids. The main cause in Dublin and in other cities of these epidemics of typhoid fever is, Sir Charles Cameron thinks, to be found in these very impure states of the soil. He says he has carefully investigated the subject and is "convinced that typhoid fever is often caused by underground air entering our dwellings" from such polluted soils.

Some years since I received a letter from Dr. Angus Mackintosh,[2] then medical officer of health of Chesterfield, in which, as a result of his experience, he professed his strong belief in the *de novo* origin of typhoid fever. He said: "If not, how can those who believe otherwise explain the mystery that enteric fever decreases in proportion as the sanitary condition of any district is improved and that as a direct consequence and in every case . . . . I say, from a lengthened experience in one of the most fever-stricken districts in England, and after carefully investigating 500 cases of that disease, in my capacity of medical officer of health, that 90 per cent. of these could not be traced by me or anybody else to a previous enteric case. The sanitary authority for which I act have borrowed £100,000 from the Loan Commissioners during the last four years for drainage works and water-supply, and by alterations and arrangements in regard to these important items they have reduced enteric fever already in the district to a very small proportion indeed."

The moral would seem to be that purity of soil is almost as important as purity of air or water; that bad drainage may pollute both air and water; and that some cases of non-specific

[1] The *Lancet*, June 11, 1892, p. 1285.
[2] Letter dated August 26, 1876.

fæcal contamination of the latter—and not only pollution by typhoid dejections—may act as causes of typhoid fever. I feel assured that these are safer and sounder doctrines than the narrower views promulgated by ultra-contagionists.

If we look to another disease or rather group of morbid conditions—namely, *Pulmonary Phthisis and Tuberculous Affections of other organs and parts*—a group so common and fatal as to constitute one of the scourges of the human race—there is room for the same uncertainty as to the proportional limits between their *de novo* origin and their spread by means of contagion, though the tendency of late has been to believe in contagion only. The great change of view that has been generally adopted by the medical profession in regard to this matter within the last few years is most remarkable and unprecedented. This change, too, has developed, not so much by reason of fresh and conclusive evidence as to the frequency of contagion in the human subject, but almost entirely from theoretical considerations and from unwillingness to believe that a disease caused by a Bacillus and capable of being freely propagated experimentally among lower animals inoculated therewith can also arise *de novo.* At the present time it is regarded as quite heretical to think it possible that phthisis can arise independently, while as late as 1896 we find Crookshank in his "Bacteriology and Infective Diseases" (p. 387) writing as follows: "Whether the disease in man is contagious is an open question, though numerous cases of supposed communication between husband and wife, brothers and sisters, have been reported, and Ransome showed that tubercle Bacilli were present in the breath in phthisis. On the other hand, the experience in consumption hospitals does not support this view, there being no evidence of the communication of the disease to nurses and hospital attendants." Such, then, have been the remarkable discrepancies in the common view entertained upon this question within a brief period of less than ten years.

At present there is a beneficent enthusiasm for "sanatoriums" in this and other European countries for the relief or the cure of patients who are afflicted with this common and very fatal affection. Phthisis is unquestionably capable of being mitigated —and even cured in many cases where it is not too far advanced —by plenty of fresh air and the best hygienic conditions.[1] This

[1] Sometimes, too, even a simple surgical operation, such as drainage of the abdomen in a case of tuberculous peritonitis, will lead to a cure of the patient, notwithstanding the presence in his tissues of untold legions of the specific Bacilli. In his admirable address to the British Medical Association at its recent meeting Mr. Mayo Robson says (*British Medical Journal*, August 1, 1903, p. 245): "I have seen patients reduced to the last extremity of weakness,

seems to me rather to lend favour to the view that it is commonly an affection produced *de novo* and altogether apart from contagion, as we formerly believed. If good hygienic conditions and improved vitality will lead to the cure of the disease, then low vitality and bad hygienic conditions may have sufficed to produce it. But, it will be said, you forget the presence of the tubercle Bacillus. To which I would reply. Have not the experiments made for the artificial production of "Pasteur's septicæmia" almost completely got over this difficulty? The injection of a small quantity of a germ-free chemical irritant into the subcutaneous tissue of a healthy rabbit has made it plain that pathogenic microorganisms may either be produced by heterogenesis in the focus of inflammation thus caused, or else that the germs of common Bacilli existing in the healthy animal on which the experiment has been made have been roused, rendered extremely virulent, and have been converted, in fact, into pathogenic Bacilli henceforth capable of acting as contagia for the indefinite propagation of this form of septicæmia. Here we have had, over and over again, in the plainest way, the *de novo* production of a contagious disease in which, as in phthisis, Bacilli act as the contagia. Why, then, should not an analogous process be similarly possible in the case of phthisis and other tuberculous affections? A Bacillus just as specific in its characters as the Bacillus tuberculosis makes its appearance also when "Davaine's septicæmia" is produced experimentally.

Half a century ago, and less, many conditions now termed "tuberculous" were then spoken of as scrofulous; and scrofula was recognised as a condition of low vitality in which inflammations of skin and mucous membranes were common, in association with enlargements of lymphatic glands in the neck and axillæ, and not infrequently chronic diseases of the joints. Now, discarding the old term, we speak of tuberculous joints and tuberculous lymphatic glands, because it is known that the specific Bacillus tuberculosis is to be found in the tissues affected, though perhaps nowhere else in the body.

But how, it may be asked, in accordance with present ultra-contagionist views as to the mode by which tuberculous affections are disseminated, are we to explain the isolated occurrence of the tubercle Bacillus within the tissues of joints, in the lymphatic glands of the axillæ, or within some of those in the neck? The two generally admitted channels for the entry of such micro-organisms into the system are through the mucous membrane of the air passages and that of the alimentary canal, but entry

where the mesentery was standing stiff with tubercle and the abdomen was swollen to an enormous size, recover completely and be thoroughly restored to perfect health from a condition apparently completely hopeless."

by either of these routes would not satisfactorily account for their isolated presence in the glands of the axillæ, or in some of those not infrequently involved in the neck, or within the tissues of the knee or hip-joints.

It has for a long time seemed to me that chronic inflammations of lymphatic glands in certain cachetic states of the system (such as we formerly labelled "scrofulous") may be of such a nature as necessarily to produce therein the little nodules which we recognise and name "tubercle." And this same view was very ably set forth in some detail by Mr. (now Sir) Frederick Treves,[1] at the International Medical Congress of 1881, in a communication entitled "Tubercle: its Histological Characters and its Relation to the Inflammatory Process, as shown in Tuberculosis of Lymphatic Glands." He pointed out that all the characters of the nodule known as "tubercle," apart from the Bacillus which had not then been discovered, can be referred to a chronic inflammatory process. He said: "The so-called tuberculous process in the external lymphatic glands can often be traced to some simple inflammatory process that implicates the radicles of the gland. Here the first change communicated to the gland is essentially inflammatory and as that change develops in the organ it begins to assume peculiar features." Of course, it would now be said, Yes, what you say may be true, but the irritative or inflammatory influence, the effects of which you recognise, is really due to, and caused by, the presence of the tubercle Bacillus. To this it may fairly be rejoined that such a view ignores the real and often obvious cause of the irritation of the gland; that it ignores the general state of the system which pre-exists and co-operates; and that it postulates infection without proof as the initial cause of the whole phenomena. But in the absence of any rational or even plausible means of accounting, in accordance with contagionist theories, for the presence of tubercle bacilli in certain lymphatic glands and in these alone, or in some joint and nowhere else in the body, it is, I think, most in harmony with existing knowledge to suppose that this particular Bacillus is a product rather than a cause of certain inflammations occurring in lowly vitalised subjects, in just the same way that the appearance of the Bacillus of "Pasteur's septicæmia" seems, as Burdon Sanderson put it, dependent "on the intensity of the inflammation itself." The adoption of such a view would go far to explain many difficulties; and the question of the etiology of tuberculous affections would be greatly simplified and brought again more closely into accord with former views.

Quite recently, however, von Behring has been advocating a very different hypothesis.[2] He believes that infection takes

[1] *Transactions of the International Medical Congress*, vol. i., pp. 298-303.

[2] *Deut. Medicin. Wochen.*, Sept. 24, 1903.

place in the main in infancy, through the intestinal canal, and that thereafter the infecting Bacilli lodging in different parts of the body commonly remain latent for years, perhaps for a long series of them. He relies in part upon recent investigations showing the high percentage of cases in which tuberculous lesions of some kind are to be found at necropsies, or demonstrated during life by tuberculin and other means; and in part upon his own researches demonstrating that tubercle Bacilli can, in infancy more especially, easily pass from the intestine into the lacteals and thence into the blood. He discredits the now commonly accepted views as to the frequency of infection through the lungs. It seems true that he has proved the possibility of infection with tubercle or other Bacilli in the manner he indicates. I, on the other hand, have proved the possibility of their *de novo* origin. He postulates long periods of latency after systematic infection, hard to be believed; and ultimately requires agencies for waking the tubercle Bacilli into activity, just such as I suppose may be adequate for calling them into being—namely, malnutrition and conditions of lowered vitality, howsoever produced, though among such factors impure air and inadequate or improper food must take an important place.

It would, of course, be an easy step to recognise the extreme probability that the conditions which had sufficed for the appearance of the characteristic Bacilli in the glands and in the joints might also obtain in the lungs. We might then return to something more like the sober views that prevailed concerning the etiology of phthisis only a few years ago when the affection was freely recognised as generable in the individual, altogether apart from contagion, and contagion was supposed to take only a limited share in the production of the disease. This seems the more rational and most warranted view to take. It is one which would tend to lay stress upon the need for prevention as well as cure, but it would not encourage the view that the disease could be exterminated, or even very largely diminished, by the provision of "sanatoriums" and by efforts to minimise the risk of contagion. I merely mean to imply that, in my opinion, contagion is as much over-rated as genesis is under-rated, and that our notions concerning prevention must not be too much centred upon the mere elimination of contagion.

I will only briefly refer to one more disease, but to one having several points of agreement with the affections last considered and concerning which much discussion has been taking place of late. I allude to that terrible affection *Leprosy*, which is generally admitted to be contagious only in a low degree and under the influence of very special conditions. Being an affection so slightly contagious and yet so widespread in different countries

the question naturally arises, Is it not also generable *de novo?* It was in the past freely believed to be so, but since 1874, when Henson discovered that a Bacillus was always to be found in the tissues affected, there has been a growing antagonism to this view owing to the yearly increasing importance of bacteriological work and the ultra-contagionist doctrines that bacteriologists seem invariably to favour.

Formerly the malady was commonly ascribed to the conjoint influence of bad hygienic surroundings, poverty, and exposure, together with deficient and improper food, more or less putrid. Of these conditions it would seem quite possible that some peculiarities in food may have been, and may still be, most potential in favouring the development of the disease. It seems to me that Mr. Jonathan Hutchinson has distinctly strengthened this view and brought forward some valuable evidence in support of his own position that badly preserved and semi-putrid fish is one of the most important factors pertaining to this category. At all events, whether his conclusions are to be accepted as correct or not, his personal researches in South Africa and in India in collecting evidence on this very important subject, involving as they must have done so great an expenditure of time and labour, are surely worthy of the highest praise.[1]

The conclusion to which he has come in regard to the relative frequency of the spread of the disease by contagion and its *de novo* origin is very similar to that arrived at by the Leprosy Commissions in India. Their verdict, given in 1890-91, was that the influence of contagion was "as small as, or even rather less than, in the case of tuberculosis,"[2] and that in the great majority of cases the disease originates *de novo*. This I regard as a perfectly logical position and one quite explicable in accordance with known facts. But it would be absolutely repudiated by many, perhaps by most, ultra-contagionists, as may be gathered from an article on Leprosy in the *Quarterly Review* for April, 1903, from the pen of a medical writer. Referring to this verdict of the Commissioners in India, and reflecting perhaps the prevailing medical opinion of to-day, this writer says (p. 397): "But did any one of the Commissioners or does anyone with knowledge of the subject contend that tuberculosis arises *de novo?* What induced the Commissioners to come to the conclusion as to the *de novo* origin of leprosy is a psychological puzzle, and it is difficult to see how the supporters of such an hypothesis account

[1] An account of his investigations will be found in the *Transactions of the Royal Medical and Chirurgical Society*, 1902, p. 161; and in the *Lancet*, May 9 (p. 1316), 23 (p. 1465), and 30 p. (1938), 1903.

[2] At that time the influence of contagion was believed to be very slight in tuberculosis.

for the presence of Bacilli in the leprous patient. Are we to suppose that these microorganisms arise spontaneously?"

In the debate which took place at the Royal Medical and Chirurgical Society, Mr. Hutchinson met with the same kind of intolerance, and there was a similar ignoring of facts which would have permitted his critics to find less difficulty in accounting for the presence of the Bacillus in a case in which leprosy had originated *de novo.* They should not have forgotten the specific Bacillus of "Pasteur's septicæmia," which can be produced at will by skilful investigators with the aid not of semi-putrid food but of a few drops of putrid blood. It was not even absolutely necessary, as I have shown in this communication, that they should believe in a *de novo* origin of the Bacillus itself; it was still less necessary that they should require its presence to be demonstrated in the bad fish or other food; and it was absurd, as I take it, gravely to attempt to shunt the real question by the gratuitous statement that "the origin of the germs of disease was probably in the remote geological past." It is a pity the able author of this sentiment did not give us some hint as to the mode of production of these germs in the past, and tell us what his warrant was for using the word "probably" in such a connexion.

What I have said in regard to the possible *de novo* origin of tuberculosis would, in fact, with slight variations be applicable in regard to leprosy. It cannot be denied that the disease is to a slight extent contagious, but there is much evidence to show that in the main it arises *de novo.* There is certainly nothing unreasonable in the supposition that putrid fish, or other bad food of like kind, may carry into the system Bacteria and the toxic products which they have formed, and that the continued influence of such bodies may, in some persons, act as irritants and either engender or awaken organisms in this or that tissue having the characteristics of the leprosy Bacillus—just as the boiled dilute liquor ammoniæ injected into the subcutaneous tissue of a guinea-pig or a rabbit produces, even within a few hours, swarms of the Bacillus met with in "Pasteur's septicæmia," or as two or three drops of putrid blood in the same situation may give rise to the appearance throughout the body of swarms of the more distinctly specific Bacilli which suffice for the indefinite propagation of "Davaine's septicæmia."

In any case the importance of diligently seeking after the cause of leprosy must be admitted, and Mr. Hutchinson is obviously right when he says[1]: "It cannot be necessary to insist that the prevention of leprosy is a work of far greater beneficence than is the mere provision for the care and comfort

[1] The *Times*, May 25, 1903.

of the leper. . . . Not only in India but in South Africa, the West Indies, and many other of our colonies, the saving in money as well as the mitigation of human suffering would be immense if the leprosy question were once settled. Large sums are now benevolently devoted to asylums for lepers. My conviction is strong that one-tenth of the sums thus annually expended would, if devoted to discovery of cause, render these establishments unnecessary and save their cost for all time." [1]

What has happened in regard to typhus fever affords the strongest testimony as to the value of the broader outlook—the search, that is, for the conditions of origin of a disease. The ravages of typhus in our crowded cities and in our jails has been enormously curtailed—not so much because of its diminished spread by contagion, but because we have learned what are the causes which engender it, and are, therefore, better able to prevent its occurrence. There can moreover be little doubt that no impassable barrier exists between non-pathogenic and pathogenic Bacteria. The mutability in form, and changeability in activity, of all these microorganisms is immense. They may merge into one another, just as the clinical types of disease with which they are associated may be united by almost insensible transitions. In a recent able communication on "The Borderlands of Diphtheria and Scarlet Fever," we find Dr. Biss, after a large experience in a fever hospital saying,[2] "the *nuances* between these conditions—scarlet fever, diphtheria, and tonsillitis—are so gentle that each shades off into the other not at one but at many points." Of course if this is true, it can only mean that there are similarly minute transitions between the activities of the microorganisms associated with the maladies in question; and that there must be the production, under certain circumstances, of "specific" from common microorganisms habitually present in the parts affected.

This would again help to bring us very much to the point of view long ago advocated by the late Professor Hueter who said at the International Medical Congress held in London in 1881: "Although it is impossible not to recognise the specific modes of activity of microorganisms in the production of infective diseases, we need not on that account deny that there is a certain unity in all these microorganisms. I am of opinion that this unity is founded upon the processes of putrefaction, and that the specific modes of activity must be regarded as depending upon certain alterations in the putrefactive process." It cannot be said that

[1] The following paragraphs are additions made to this article since it appeared in the columns of *The Lancet*.

[2] *The Lancet*, Nov. 7, 1903, p. 1291.

the vast mass of subsequent investigations have displaced such a view, seeing that no less an authority than Prof. Hueppe, of Prague in his first "Harben Lecture," recently delivered in this city, is reported to have said existing evidence favoured the view that the origin of all common infectious diseases was "phylogenetically traceable to putrefactive processes."[1]

Let us then strive to ascertain the conditions of origin of all contagious affections. The more contagious they are, the more important does the quest become. Let us not blindly think that contagion is the one and only cause, but seek in all doubtful and obscure cases, and by cumulation of evidence, to ascertain what are the invariable and immediately antecedent sets of conditions, or states of system, that may have sufficed to engender this or that contagious disease. Progress, however slow, may in this way ultimately reward our efforts, and we may gradually attain a knowledge that will confer great power in checking the ravages of these pestilential affections—a power to which we shall never attain so long as we pin our faith exclusively to the narrower ultra-contagionist doctrines now so prevalent.

[1] *The Lancet*, Oct. 31, p. 1217.

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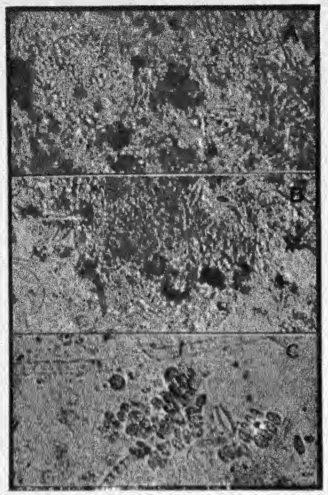

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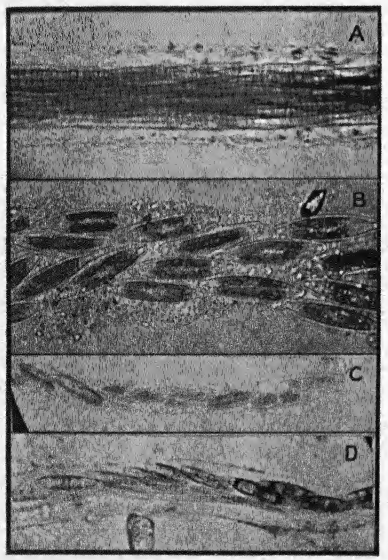

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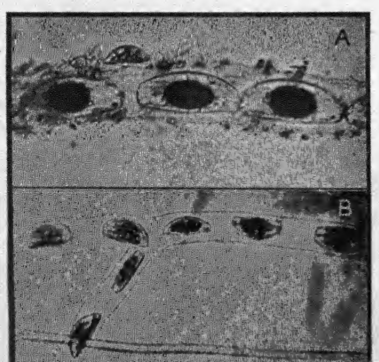

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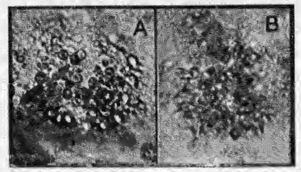

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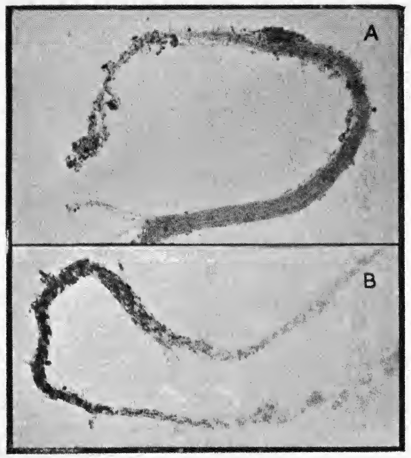

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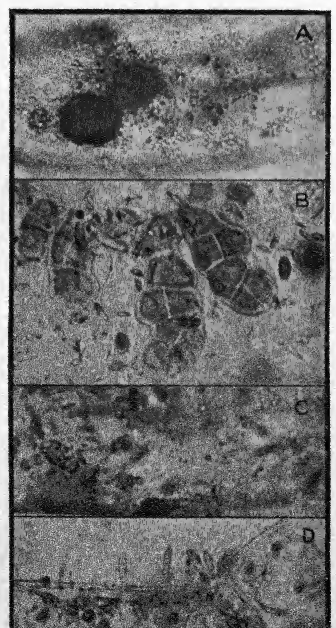

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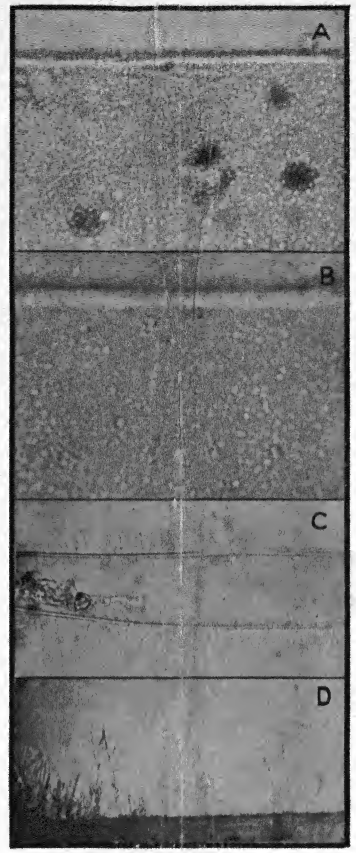

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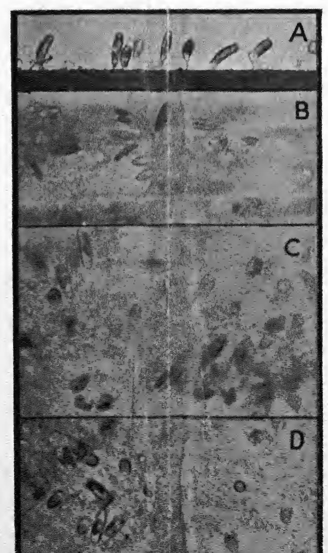

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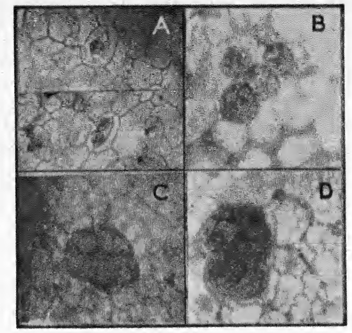

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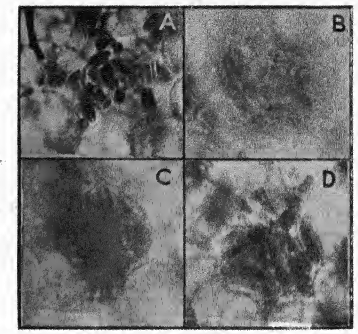

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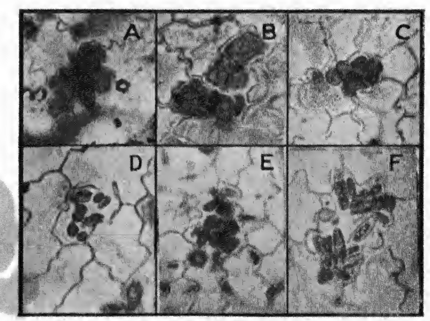

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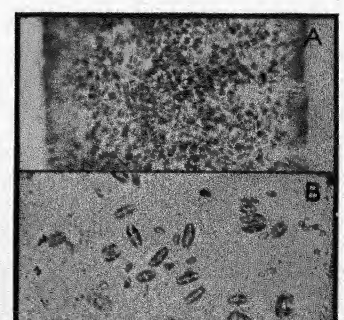

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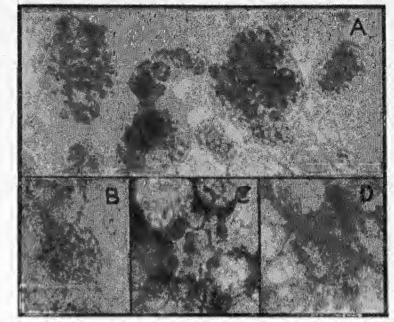

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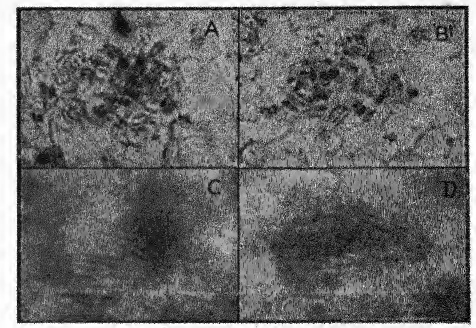

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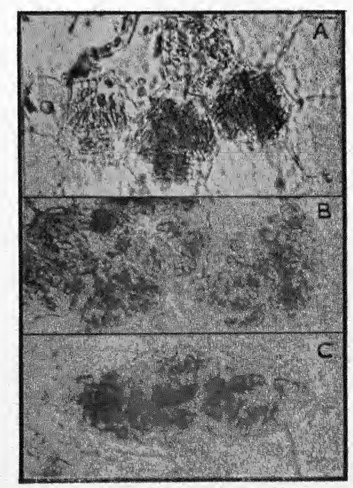

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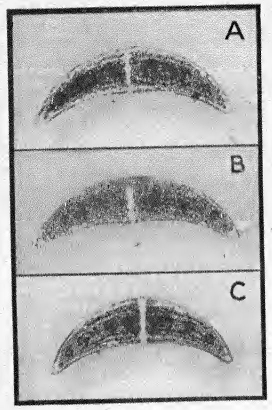

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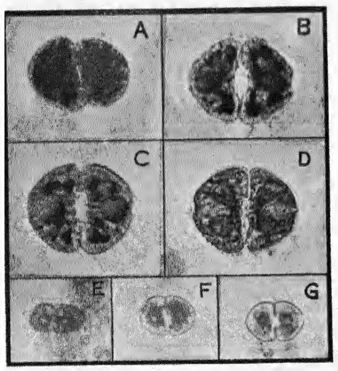

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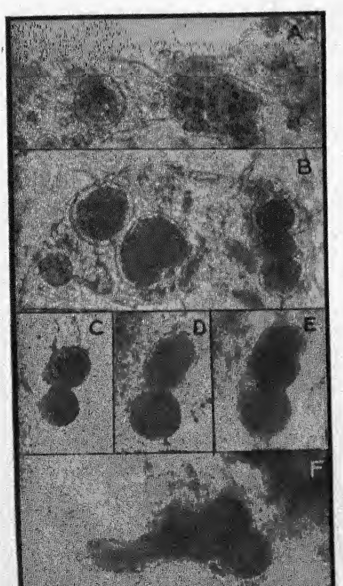

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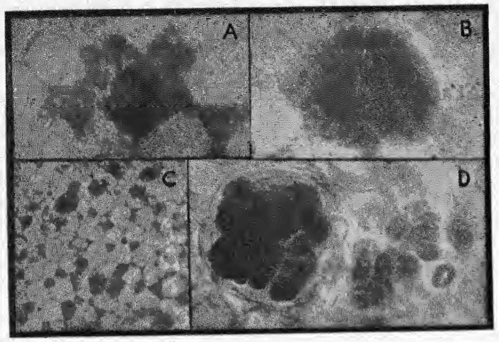

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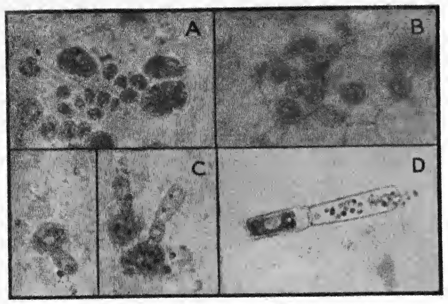

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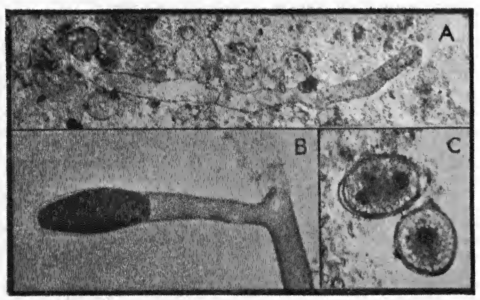

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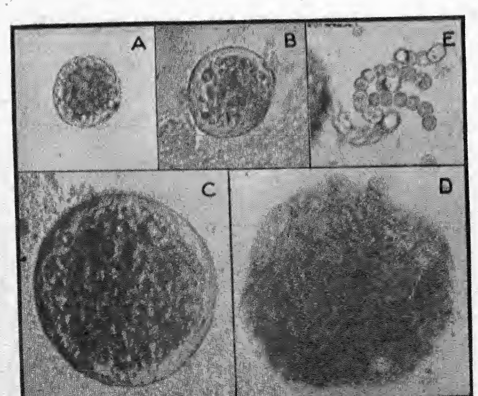

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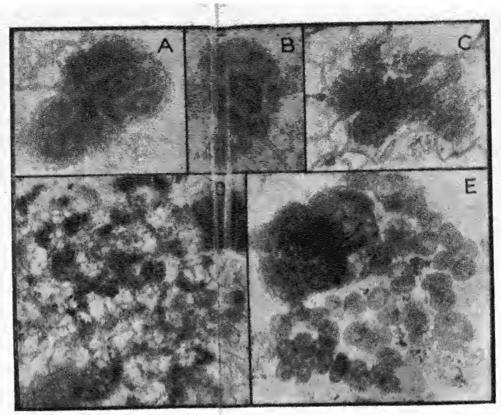

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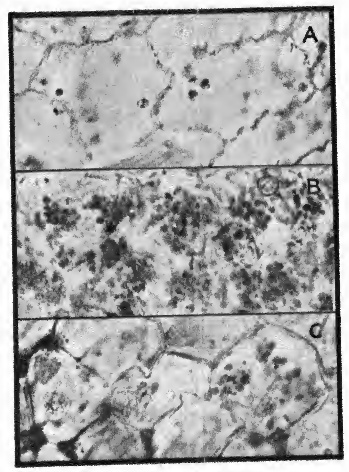

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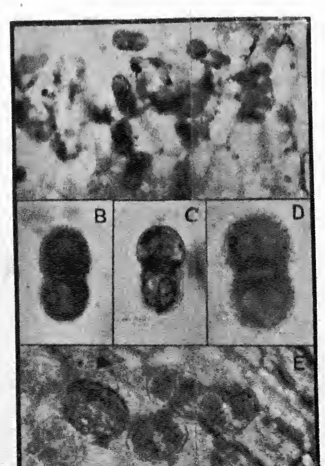

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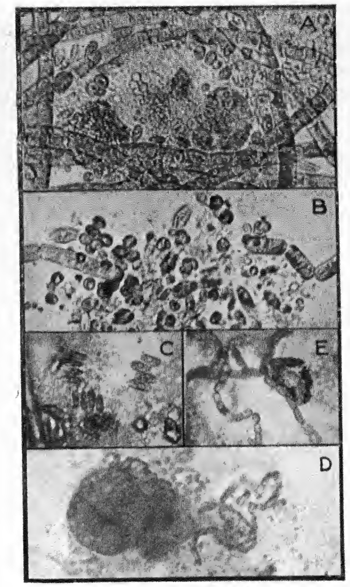

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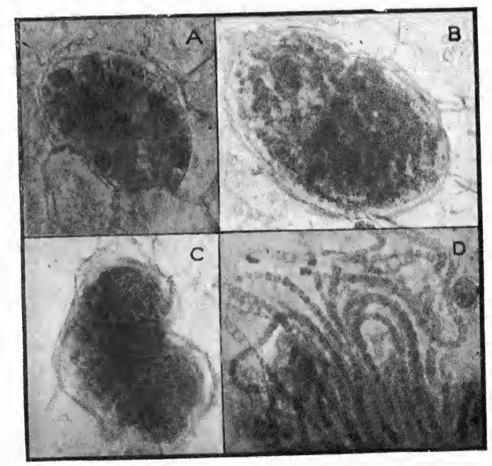

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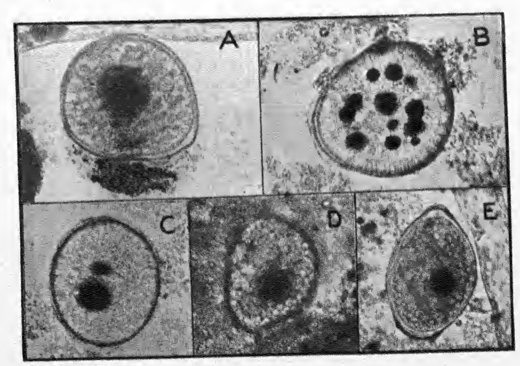

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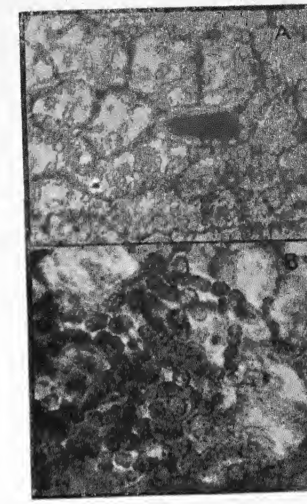

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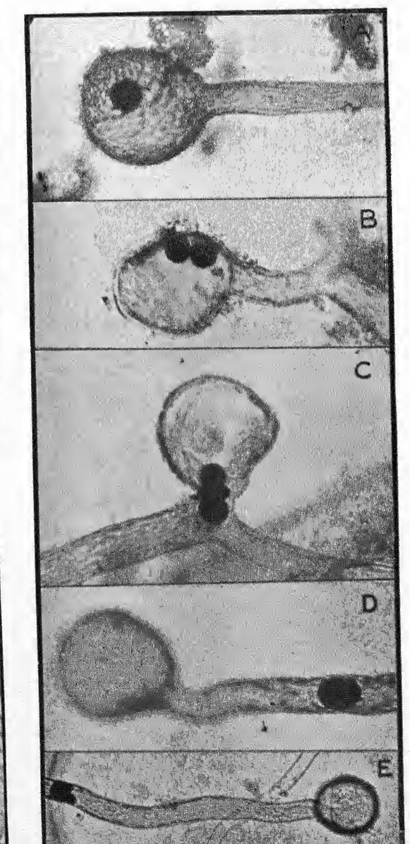

Fig. 139. p. 199.

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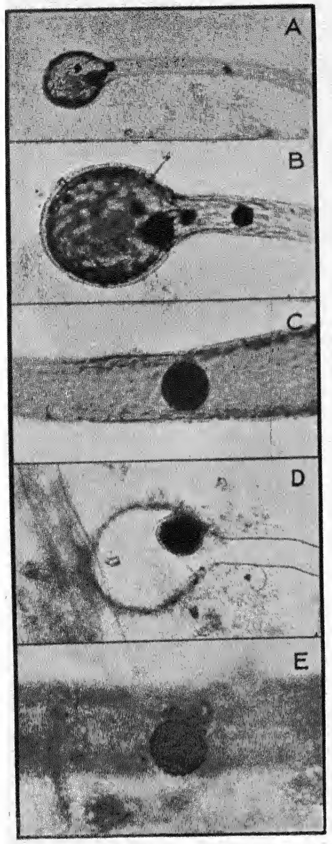

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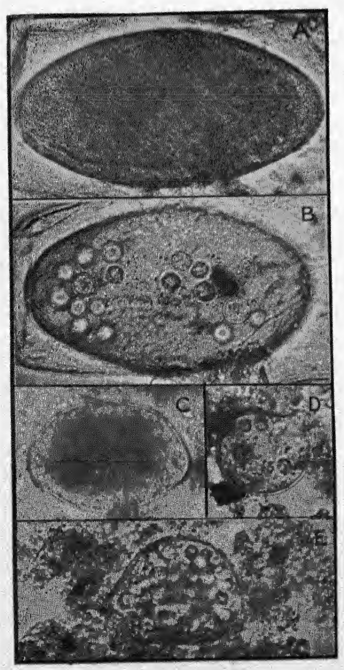

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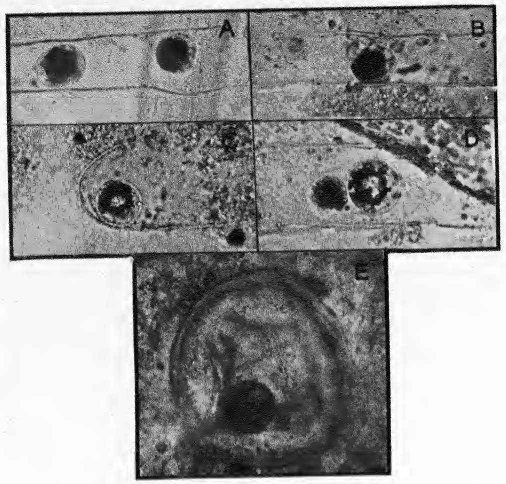

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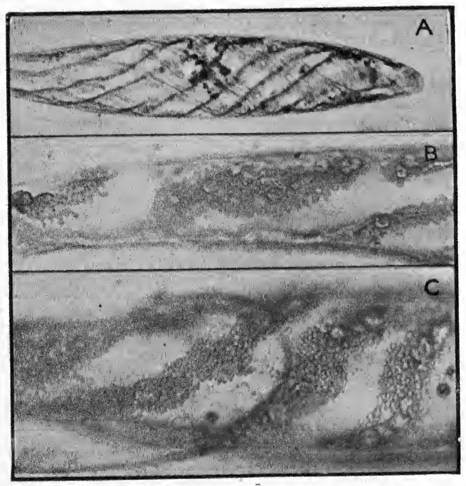

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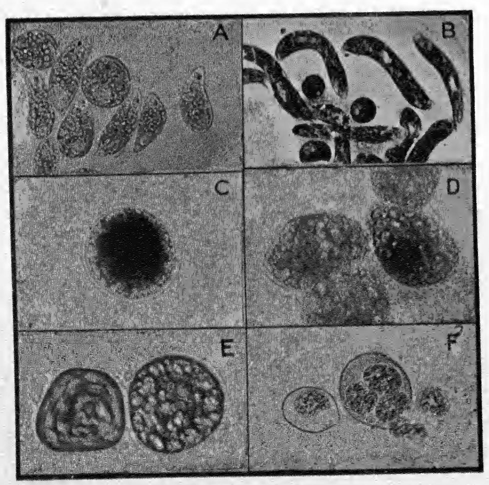

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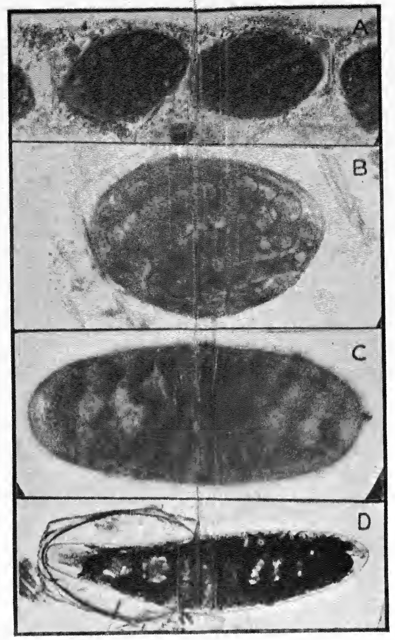

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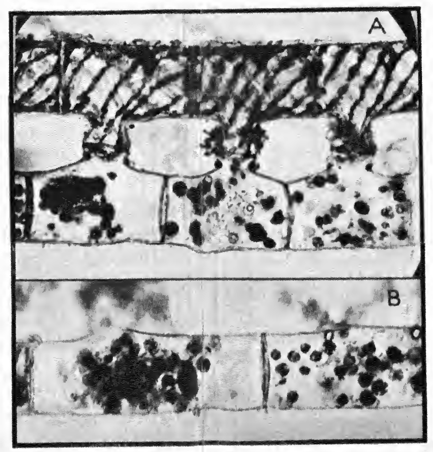

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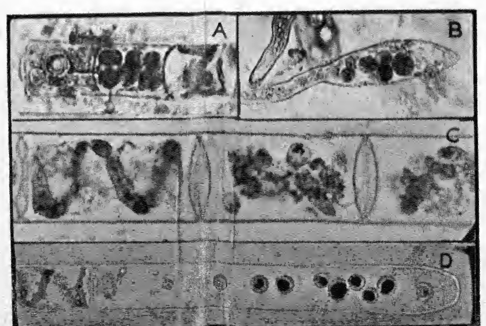

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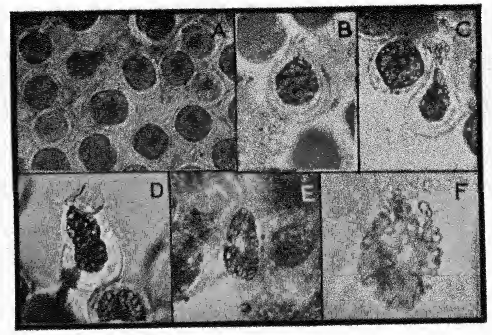

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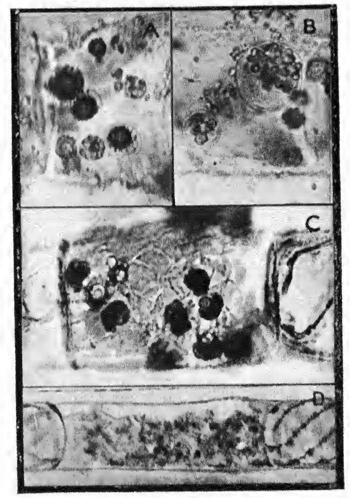

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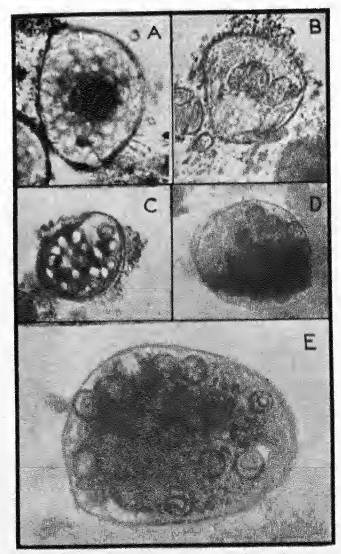

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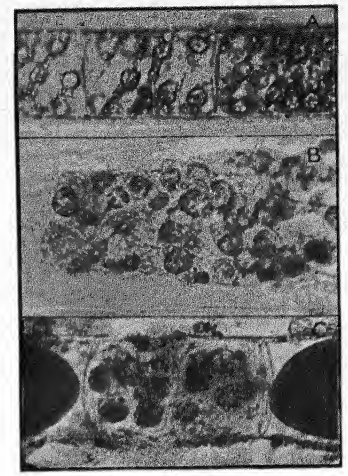

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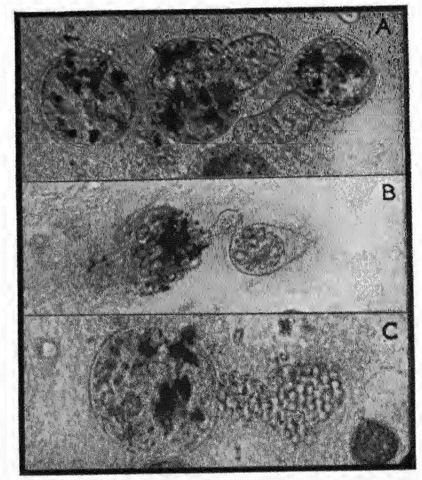

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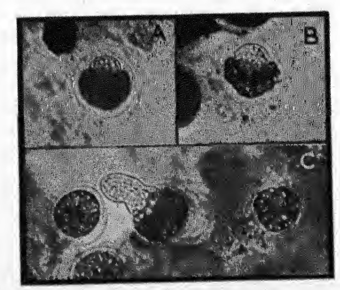

Fig. 151. p. 224.

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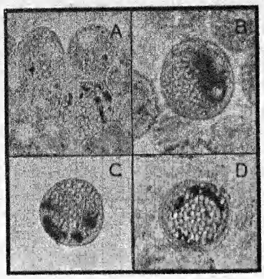

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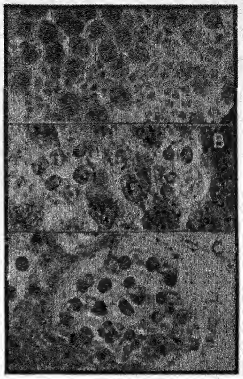

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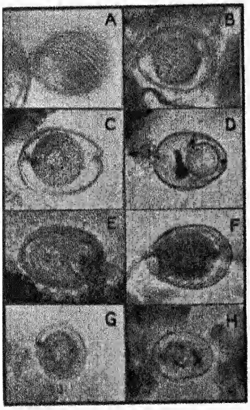

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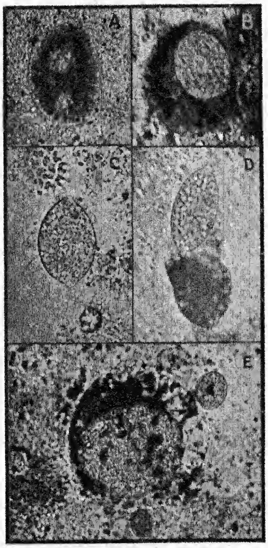

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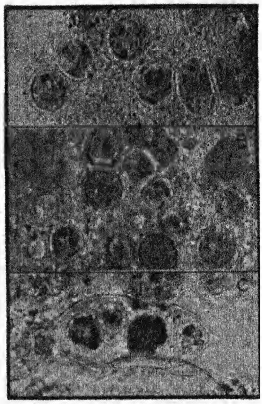

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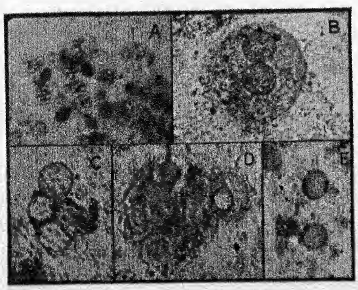

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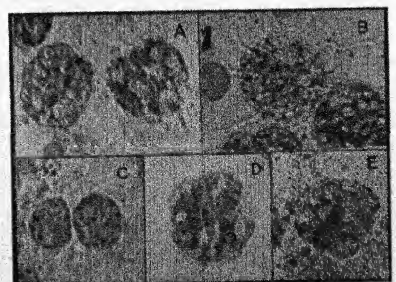

Fig. 157. p. 233.

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PLATE XVI.

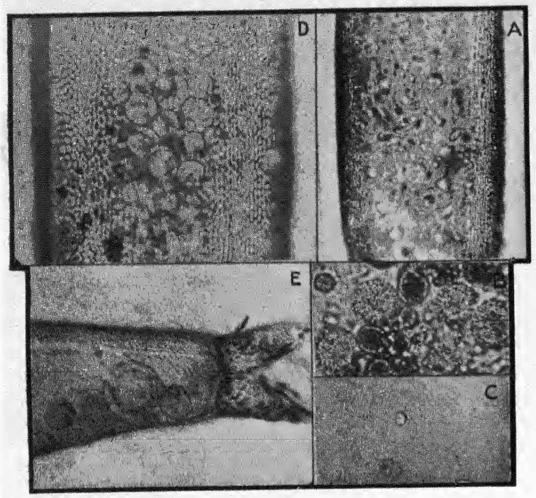
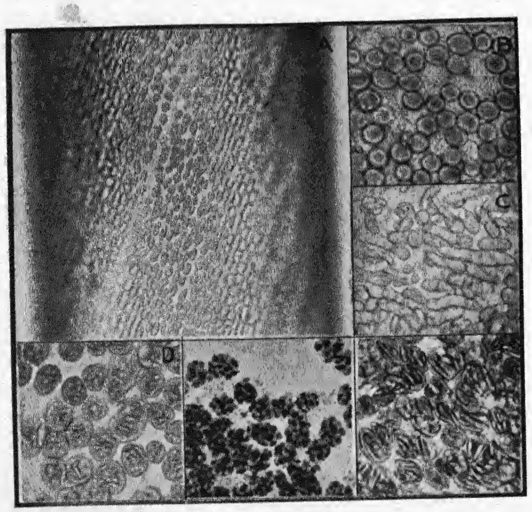


Fig. 160. p. 246.



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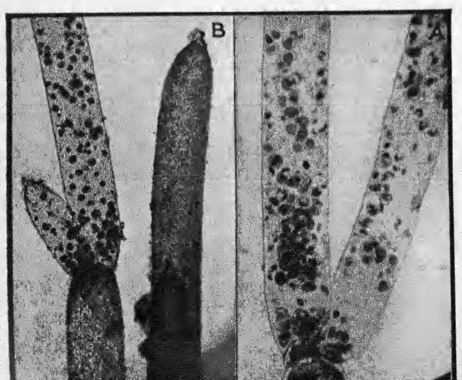
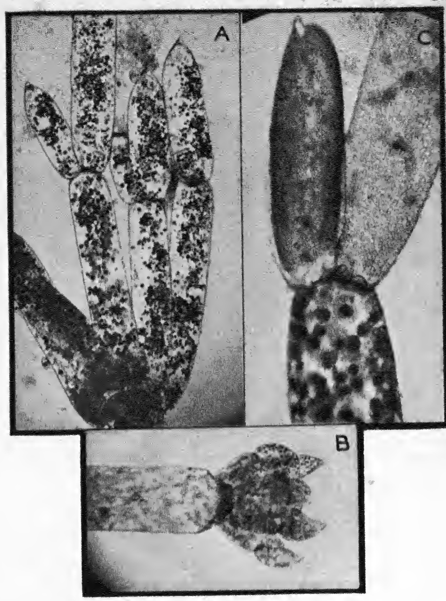


Fig. 163. pp. 248, 261.



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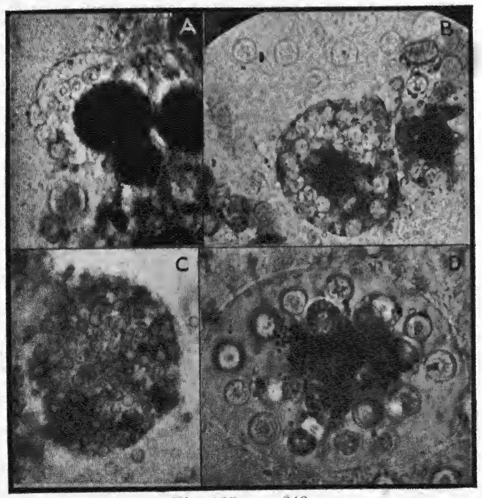

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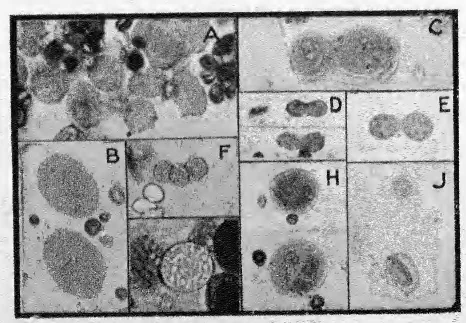
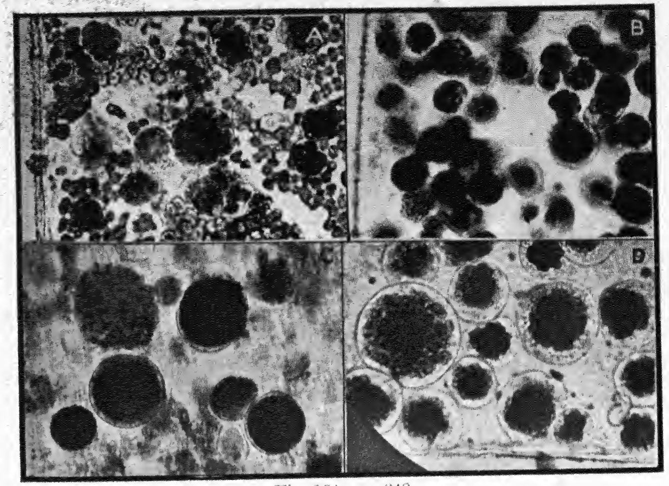


Fig. 168. pp. 256, 259.



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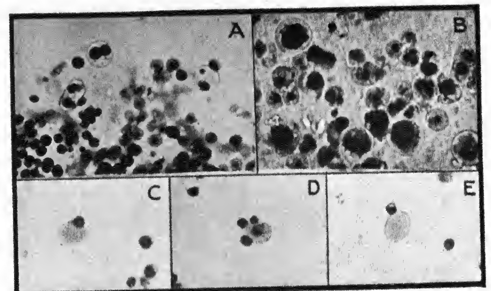

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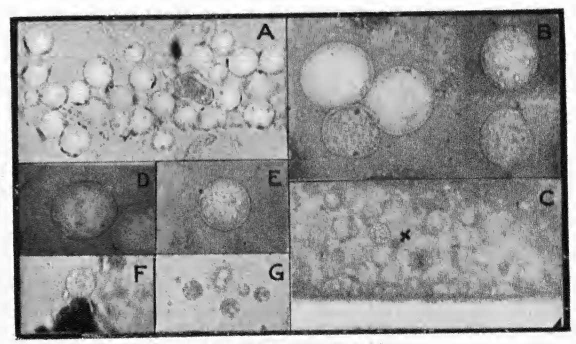


Fig. 168a. pp. 246, 260.

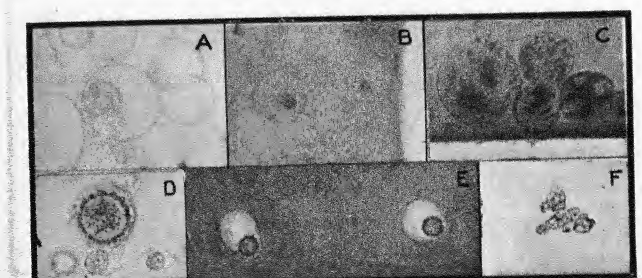
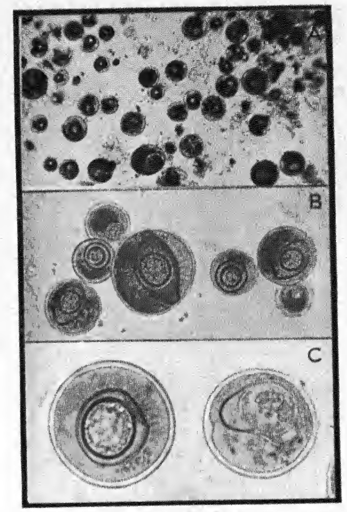


Fig. 168b. pp. 246, 260.



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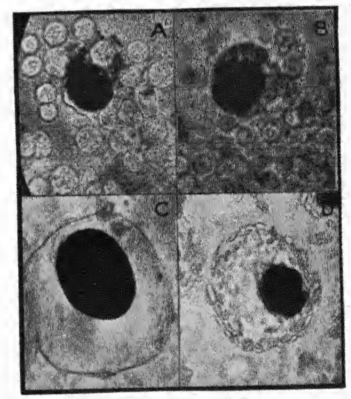

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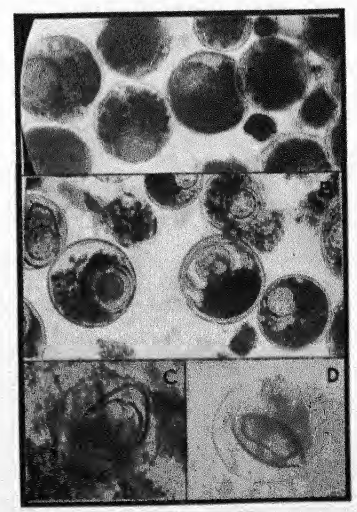

Fig. 170. p. 262.

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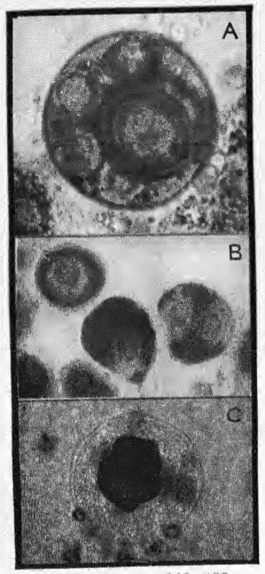
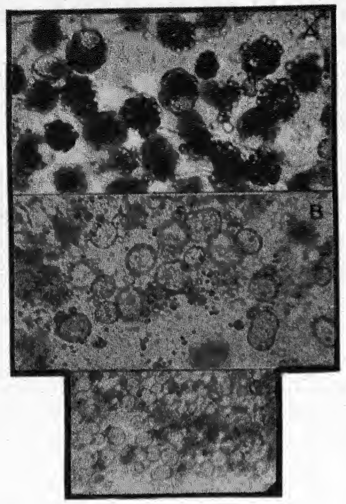


Fig. 171. pp. 262, 265.



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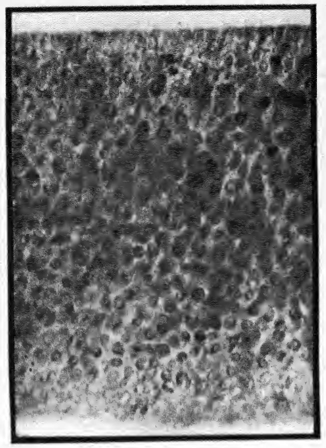
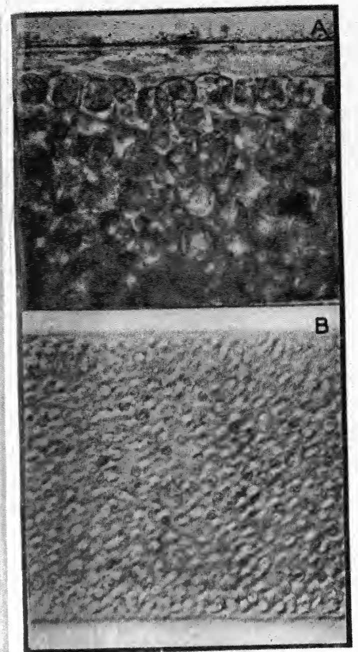


Fig. 174. p. 273.



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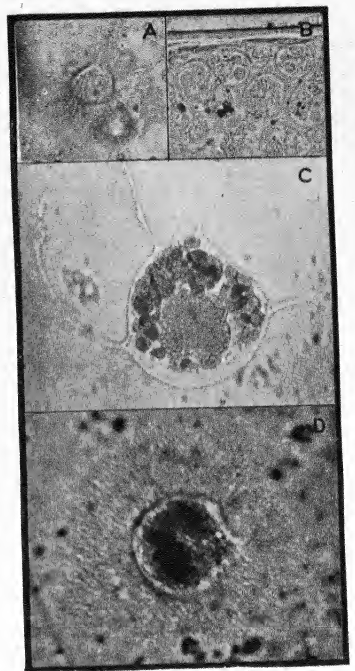

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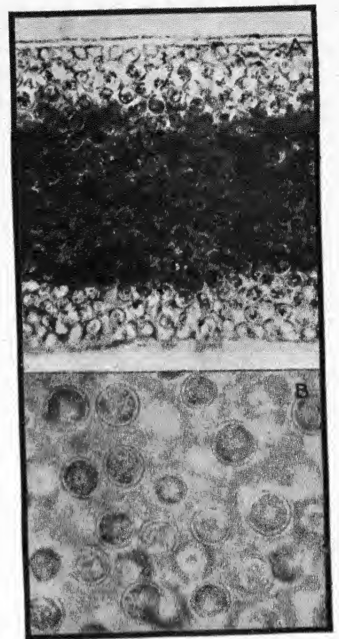


Fig. 176. p. 273.

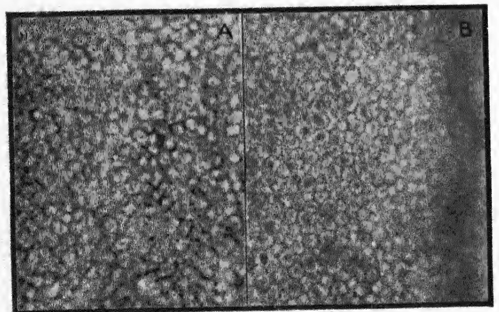


Fig. 172. pp. 266, 272.

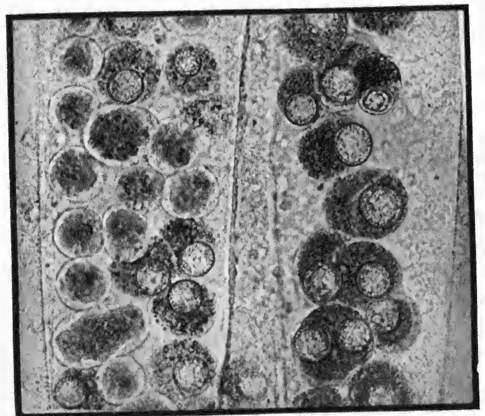


Fig. 180. p. 277.

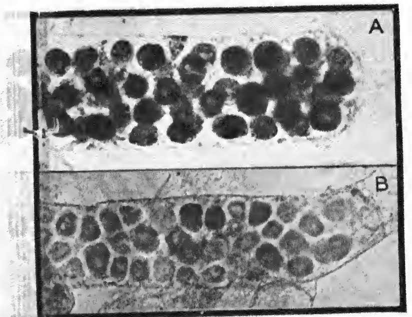
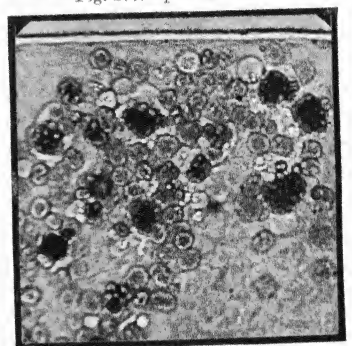


Fig. 182. p. 278.



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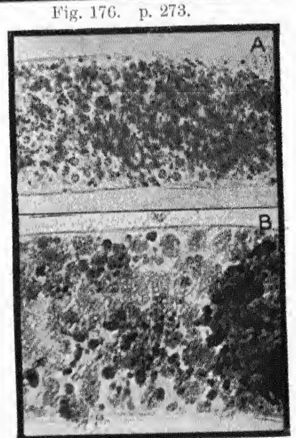

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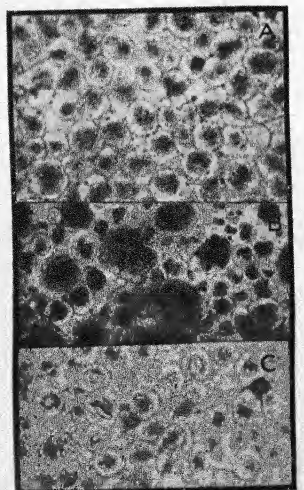
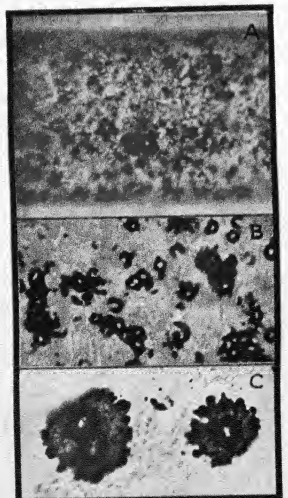


Fig. 172a. p. 268.



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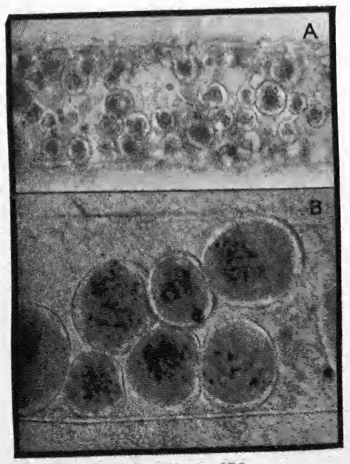

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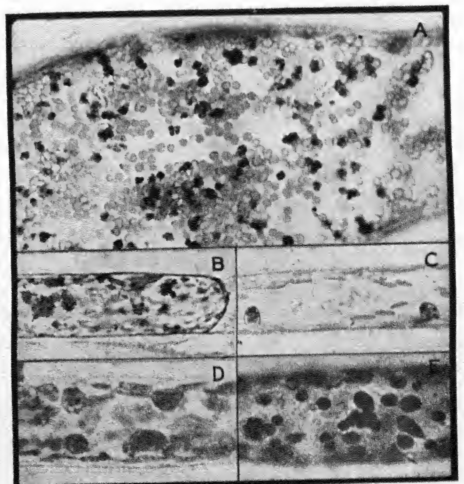

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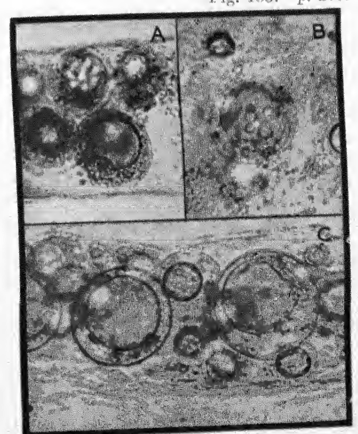

Fig. 181. p. 278.

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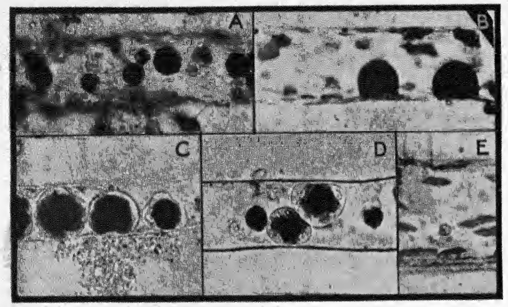

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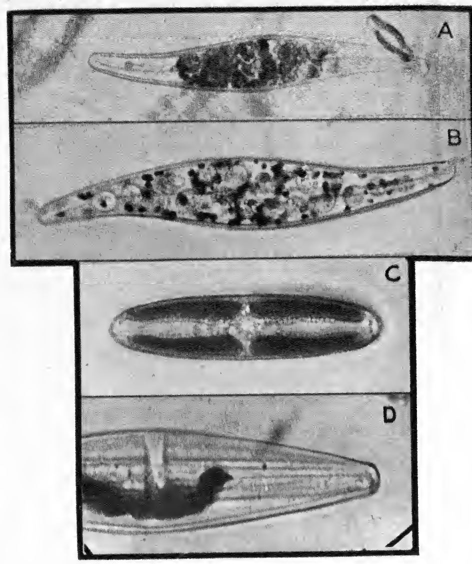

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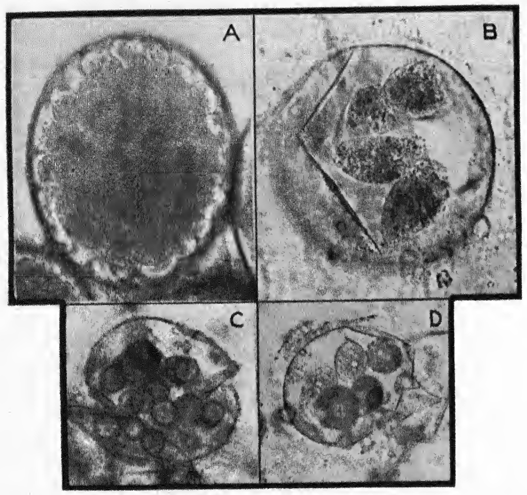

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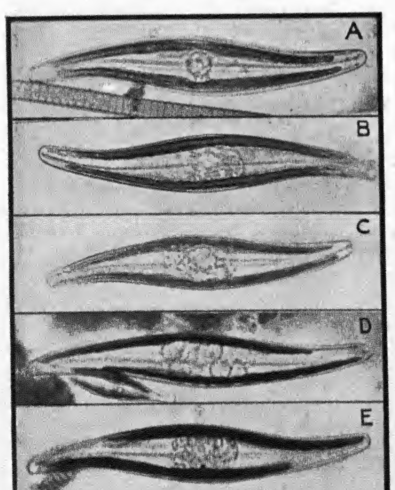

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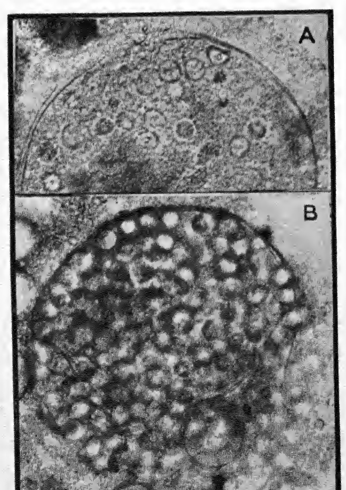

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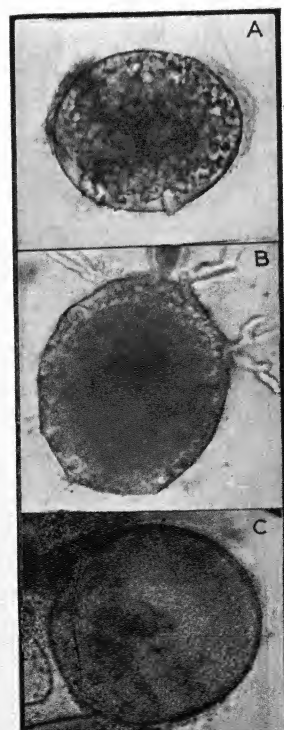

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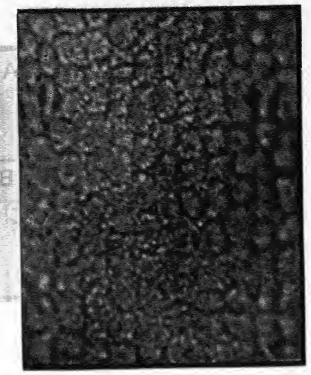
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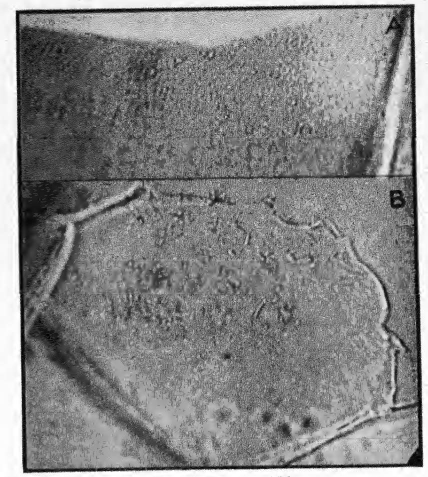

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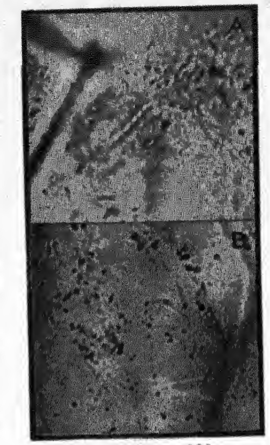

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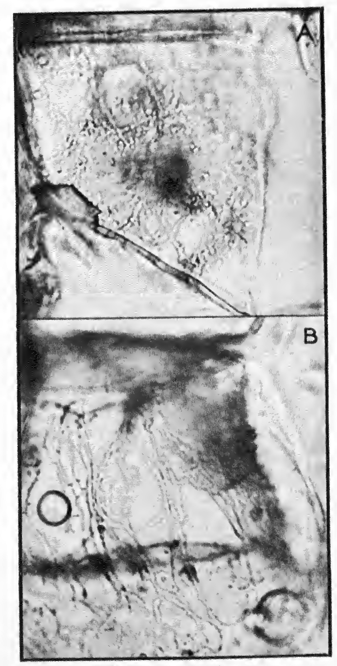

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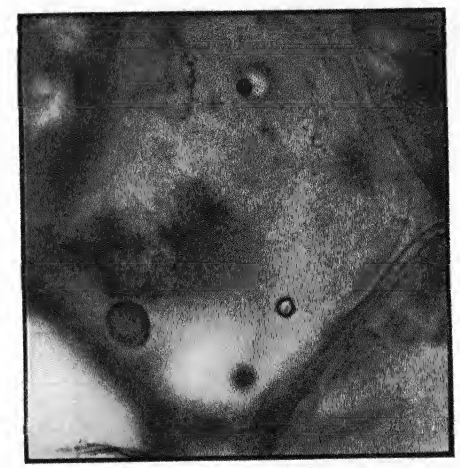
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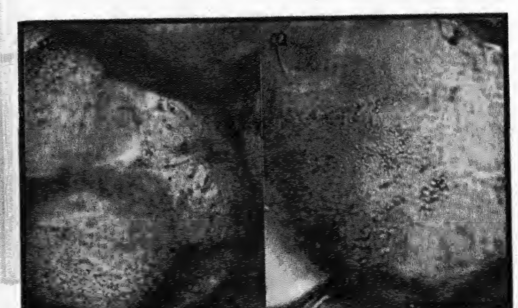
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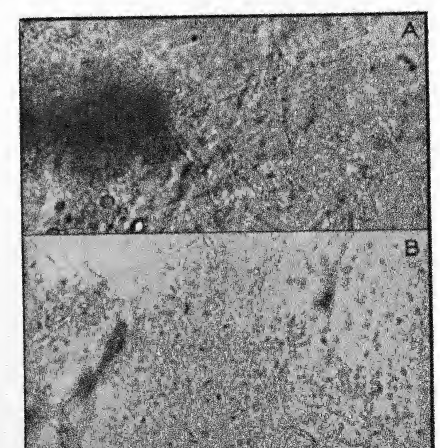

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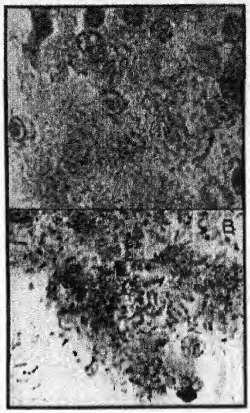

Fig. 201. p. 310.

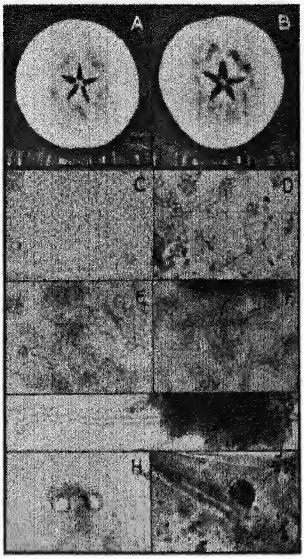

Fig. 199. p. 304.

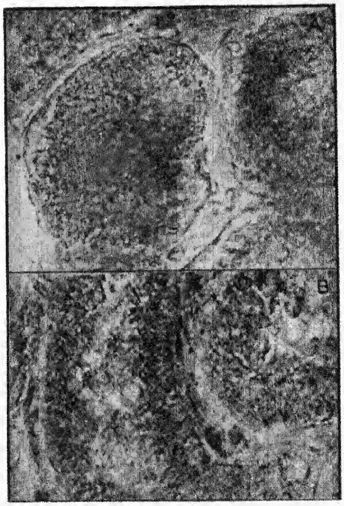

Fig. 202. p. 311.

Fig. 200. p. 307.

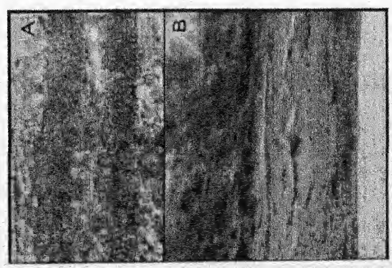

Fig. 203. p. 311.

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